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Methods for the Determination of Organic Compounds in Drinking Water. Volume 2

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Abstract: Nine analytical methods covering 54 organic contaminants which may be present in drinking water or drinking water sources are described in detail. Seven of these methods cover compounds designated for regulation under the Safe Drinking Water Act Amendments of 1986. Regulations for the group are in the proposal stages with promulgation scheduled for June 1992. The other two methods are for chlorination disinfection byproducts and may be regulated as part of EPA's disinfectants and disinfectant byproducts rule scheduled for proposal early in 1992. Most of the analytes may be classified as non-volatile and three of the methods entail separations by high performance liquid chromatography. The remainder employ capillary column gas chromatography. One of these requires detection of a potentially very toxic contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin, at the low parts per trillion level. Labeled isotopes of the analyte are employed as tracers and mass spectrometry is required for detection and unambiguous identification. Three of the methods herein offer new and simplified liquid-solid extraction procedures, a trend which is likely to become even more pronounced in the future.

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METHODS FOR THE DETERMINATION OF ORGANIC COMPOUNDS IN DRINKING WATER

VOLUME II

Environmental Monitoring Systems Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring Systems Laboratory - Cincinnati (EMSL-Cincinnati) conducts research to:

- o Develop and evaluate analytical methods to identify and measure the concentration of chemical pollutants in drinking waters, surface waters, groundwaters, wastewaters, sediments, sludges, and solid wastes.
- o Investigate methods for the identification and measurement of viruses, bacteria and other microbiological organisms in aqueous samples and to determine the responses of aquatic organisms to water quality.
- o Develop and operate a quality assurance program to support the achievement of data quality objectives in measurements of pollutants in drinking water, surface water, groundwater, wastewater, sediment and solid waste.

This publication of the Environmental Monitoring Systems Laboratory - Cincinnati titled, "Determination of Organic Compounds in Drinking Water Volume II" was prepared to gather together under a single cover a set of 9 laboratory analytical methods for organic compounds in drinking water. We are pleased to provide this manual and believe that it will be of considerable value to many public and private laboratories that wish to determine organic compounds in drinking water for regulatory or other reasons.

Thomas Clark, Director
Environmental Monitoring Systems
Laboratory - Cincinnati

ABSTRACT

Nine analytical methods covering 54 organic contaminants which may be present in drinking water or drinking water sources are described in detail. Seven of these methods cover compounds designated for regulation under the Safe Drinking Water Act Amendments of 1986. Regulations for this group are in the proposal stages with promulgation scheduled for June 1992. The other two methods are for chlorination disinfection byproducts and may be regulated as part of EPA's disinfectants and disinfectant byproducts rule scheduled for proposal early in 1992. Most of the analytes may be classified as non-volatile and three of the methods entail separations by high performance liquid chromatography. The remainder employ capillary column gas chromatography. One of these requires detection of a potentially very toxic contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin, at the low parts per trillion level. Labeled isotopes of this analyte are employed as tracers and mass spectrometry is required for detection and unambiguous identification. Three of the methods herein offer new and simplified liquid-solid extraction procedures, a trend which is likely to become even more pronounced in the future.

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ANALYTE - METHOD CROSS REFERENCE

Analyte	Method No.
Acenaphthene	550, 550.1
Acenaphthylene	550, 550.1
Anthracene	550, 550.1
Benzo(a)anthracene	550, 550.1
Benzo(b)fluoranthene	550, 550.1
Benzo(k)fluoranthene	550, 550.1
Benzo(g,h,i)perylene	550, 550.1
Benzo(a)pyrene	550, 550.1
Bis(2-ethylhexyl)adipate	506
Bis(2-ethylhexyl)phthalate	506
Bromochloroacetic Acid	552
Bromochloroacetonitrile	551
Bromodichloromethane	551
Bromoform	551
Butylbenzyl phthalate	506
Carbon Tetrachloride	551
Chloral Hydrate	551
Chloroform	551
Chloropicrin	551
Chrysene	550, 550.1
Dibenzo(a,h)anthracene	550, 550.1
Dibromoacetic Acid	552
Dibromoacetonitrile	551
Dibromochloromethane	551
1,2-Dibromo-3-chloropropane(DBCP)	551
1,2-Dibromoethane(EDB)	551
Dichloroacetic Acid	552
Dichloroacetonitrile	551
2,4-Dichlorophenol	552
1,1-Dichloropropanone-2	551
Diethyl phthalate	506
Dimethyl phthalate	506
Di-n-butyl phthalate	506
Di-n-octyl phthalate	506
Diquat	549
Endothall	548
Fluoranthene	550, 550.1
Fluorene	550, 550.1
Glyphosate	547
Indeno(1,2,3-cd)pyrene	550, 550.1
Monobromoacetic Acid	552
Monochloroacetic Acid	552

Analyte	Method No.
Naphthalene	550, 550.1
Paraquat	549
Phenanthrene	550, 550.1
Pyrene	550, 550.1
2,3,7,8-Tetrachlorodibenzo-p-dioxin	513
Tetrachloroethylene	551
Trichloroacetic Acid	552
Trichloroacetonitrile	551
1,1,1-Trichloroethane	551
Trichloroethylene	551
2,4,6-Trichlorophenol	552
1,1,1-Trichloropropanone-2	551

INTRODUCTION

Jimmie W. Hodgeson

An integral component of the role of the Environmental Protection Agency (EPA) in protecting the quality of the Nation's water resources is the provision of means for monitoring water quality. In keeping with this role, EPA develops and disseminates analytical methods for measuring chemical and physical parameters affecting water quality, including chemical contaminants which may have potential adverse effects upon human health. This manual provides nine, analytical methods for 54 organic contaminants, which may be present in drinking water or drinking water sources. In December 1988, EPA published "METHODS FOR THE DETERMINATION OF ORGANIC COMPOUNDS IN DRINKING WATER", a manual containing 13 methods for approximately 200 potential drinking water contaminants. The current manual is a complement to the earlier version, providing for the most part methods for analytes which appear at a later time in the regulatory framework. Efforts have been made herein to provide a manual and methods format, which is consistent with the earlier version.

REGULATORY BACKGROUND

Analytical methodology for monitoring water quality serve a number of related purposes, including occurrence studies in community water systems, health effects studies and the determination of the efficacy of various water treatment approaches. These activities, in turn, form the supporting bases for water quality regulations, and the support of these regulations is the ultimate purpose of the analytical methods. Limitations on the levels of specific contaminants are codified in proposed and promulgated Federal regulations developed in response to the Safe Drinking Water Act (SDWA) of 1974 and the SDWA amendments of 1986. The Act requires EPA to promulgate regulations for contaminants in drinking water which may cause adverse health effects and which are known or anticipated to occur in public water systems. The 1986 amendments require regulations to include Maximum Contaminant Levels (MCL's) with compliance determined by regulatory monitoring or by the application of an appropriate treatment, when adequate analytical methodology is not available. In addition, the 1986 amendments specified 83 contaminants, originally scheduled for regulation by June 19, 1989. The amended Act also required EPA to develop a priority list of additional contaminants, to propose 25 more of these by January of 1988 for subsequent regulation and to continue this process by the addition of 25 from the priority list on a triennial basis thereafter.

Of the original 83 pollutants, regulations for eight volatile organic chemicals (VOC) were promulgated in June 1987 (see 52 FR 25690 and 51 FR 11396). Analytical methods for these eight as well as other unregulated VOC's were published in the December 1988 manual (EPA Methods 502.1, 502.2, 503.1, 524.1 and 524.2). Regulations for thirty synthetic organic chemicals (SOC's) were proposed July 22, 1989 (54 FR 22062) and scheduled for promulgation by December 1990. Note that this group included six SOC's which, by authority of provisions in the 1986 amendments, were substituted into the original list of 83 in January 1988 (53 FR 1892) - namely aldicarb sulfoxide, aldicarb sulfone, ethylbenzene, heptachlor, heptachlor epoxide and styrene. With the exception of lindane, analytical methods for all thirty compounds are by the VOC methods above or SOC methods also included in the 1988 manual.

The current manual provides analytical methods for many of the remaining contaminants on the original list of 83 - namely adipates, diquat, endothall, glyphosate, polycyclic aromatic hydrocarbons (PAH's), phthalates and dioxin. Phase V of EPA regulations for these and eleven other remaining SOC's from the list of 83 is scheduled for proposal in June 1990 and promulgation in March 1992. Analytical methods for the latter eleven were included in the December 1988 manual. Methods 551 and 552 of this manual cover the most important classes of organic chlorination disinfection byproducts. These contaminants were included in the first EPA priority list of additional substances, which may require regulation under the Act (see 53 FR 1892). At least some of these will be regulated by EPA's phase IV disinfectants and disinfectant rule scheduled for proposal early in 1992.

GENERAL COMMENTS

The current manual provides methods, which are in the same format and cast in the same terminology as the December 1988 manual. The introduction to the earlier manual discusses general method features on format, sample matrices, method detection limits and calibration and quality control samples. These same comments apply herein. In particular, these methods are written in standardized terminology in a stand-alone format, requiring no other source material for application. The methods are designed for drinking water and drinking water sources and not for more complex matrices such as waste water, hazardous waste effluents or biological fluids. The method detection limits provided were determined by replicate analyses of fortified reagent water over a relatively short period of time. As such, these are somewhat idealized limits, but nevertheless provide a useful index of method performance. Reporting limits for reliable quantitative data may be considerably higher.

The quality assurance sections are uniform and contain minimum requirements for operating a reliable monitoring program - initial demonstration of performance, routine analyses of reagent blanks, analyses of fortified reagent blanks and fortified matrix samples and analyses of quality control (QC) samples. Other quality control practices are recommended and may be adopted to meet the particular needs of monitoring programs - e. g., the analyses of field reagent blanks, instrument control samples and performance evaluation samples. Where feasible, surrogate analytes have been included in the methods as well as internal standards for calibration. Surrogate recoveries and the internal standard response should be routinely monitored as continuing checks on instrument performance, calibration curves and overall method performance.

THE ANALYTICAL METHODS

This manual includes seven methods for synthetic organic chemicals and two methods for chlorination disinfection byproducts. In general, the analytes may by classified as non-volatile and three of the methods employ separation by high performance liquid chromatography (HPLC). The remainder utilize capillary column gas chromatography (GC). Two of the methods use convenient liquid-solid extraction (LSE) methods for analyte isolation and a third offers LSE as an option. By contrast to the original manual, four of the methods are for single analytes - 2,3,7,8-tetrachlorobenzo-p-dioxin, glyphosate, endothall and diquat. These analytes are not readily amenable to generic methods. Each method provides an adequate summary statement. Some additional comments are germane here.

Method 506 for phthalates and adipates offers both liquid-liquid extraction (LLE) and LSE options. After capillary column GC separation, a photoionization detector is required for detection and MDL's are limited to approximately 10 µg/L. Phthalates and adipates are among the most common contaminants encountered in the laboratory and extreme care must be taken to ensure clean reagent blanks.

Dioxin may be an extremely toxic chemical and water concentrations of a few parts per trillion (pg/L) are of concern. In addition to extreme sensitivity, unambiguous identification is an analytical requirement. Thus, Method 513 is an inherently complex method, which employs LLE, extract cleanup, a sample concentration factor of 10^5, capillary column GC separation and analysis by mass spectrometry. In addition, labeled isotopes of dioxin are employed as surrogate analyte and internal standard to aid in identification and quantitation and to compensate for analyte losses during the complex sample handling procedure.

Methods 547, 548 and 549 are single analyte procedures for glyphosate, endothall and diquat. Paraquat is a non-regulated ionic herbicide quite similar to diquat and may be analyzed simultaneously. These may be characterized as difficult analytes because of their high water solubility and low volatility. In addition, glyphosate and endothall require derivatization prior to detection. Glyphosate is analyzed by direct aqueous HPLC injection and undergoes post-column derivatization prior to fluorescence detection. Endothall must be transferred from the aqueous phase to an acetic acid matrix for derivatization, followed by analysis by GC with electron capture detection (ECD). As with glyphosate, no preconcentration factor is involved. Method 549 provides for the extraction and concentration of diquat and paraquat in the base forms by LSE with a C-8 cartridge, preconditioned to operate in the reverse phase mode. The analytes are eluted with an acidic solvent and, after addition of ion-pairing reagent, are separated by HPLC. The analytes are detected by ultraviolet absorption (UV) with confirmation provided by a photodiode array spectrometer.

Methods 550 and 550.1 provide HPLC alternatives to the GC-MS procedure of method 525 in the December 1988 manual. Method 550 employs a conventional serial LLE approach while 550.1 uses a LSE procedure similar to Method 525. Dual UV and fluorescence detectors are employed, with considerably lower MDL's reported for the latter.

Halogenated organic byproducts, other than the regulated trihalomethanes, account for most of the total organic halogen formed by the chlorination of water supplies. The most important classes in terms of occurrence are the neutral analytes of Method 551 and the haloacetic acids of Method 552. Method 551 is quite similar to Method 504, employing a simple one step LLE and direct injection of the extract into a capillary GC with ECD detection. The haloacetic method is a serial LLE with analysis by GC-ECD and is quite similar to, but simpler than, Method 515.1 for acid herbicides. Both employ diazomethane for methylation with a micromolar generation procedure, which avoids the hazards associated with handling concentrated diazomethane. These two methods are unique in that significant background concentrations will always be present in chlorinated supplies. When determining fortified matrix recoveries as required in the quality assurance (QA) section, these background levels must be taken into account when deciding upon fortification concentrations. In addition, the uncertainty in measuring the background level should be considered when establishing control limits, as called for in the QA section.

METHOD 506. GAS CHROMATOGRAPHIC DETERMINATION OF PHTHALATE AND ADIPATE ESTERS IN DRINKING WATER

July 1990

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METHOD 506

GAS CHROMATOGRAPHIC DETERMINATION OF PHTHALATE AND ADIPATE ESTERS IN DRINKING WATER

1. SCOPE AND APPLICATION

1.1 This method describes a procedure for the determination of certain phthalate and adipate esters in drinking water by liquid/liquid or liquid/solid extraction. The following analytes can be determined by this method:

PARAMETER	STORET NO.	CAS NO.
Bis (2-ethylhexyl) phthalate	39100	117-81-7
Butylbenzyl phthalate	34292	85-68-7
Di-n-butyl phthalate	39110	84-74-2
Diethyl phthalate	34336	84-66-2
Dimethyl phthalate	34341	131-11-3
Bis(2-ethylhexyl) adipate	--	103-23-1
Di-n-octyl phthalate	--	117-81-7

1.2 This is a capillary column gas chromatographic (GC) method applicable to the determination of the compounds listed above in ground water and finished drinking water. When this method is used to analyze unfamiliar samples for any or all of the compounds listed above, compound identifications should be supported by at least one additional qualitative technique. Method 525 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for all the analytes listed above, using the extract produced by this method.

1.3 This method has been validated in a single laboratory and method detection limits (MDLs) (1) have been determined for the analytes above (Table 2). Observed detection limits may vary among waters, depending upon the nature of interferences in the sample matrix and the specific instrumentation used.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of GC and in the interpretation of gas chromatograms obtained by a computerized system. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Sect. 10.

2. SUMMARY OF METHOD

2.1 A measured volume of sample, approximately 1-L, is extracted with a ternary solvent consisting of methylene chloride, hexane and ethyl acetate using a glass separatory funnel. The solvent extract is isolated, dried and concentrated to a volume of 5 mL or less. The extract is further concentrated by gentle use of nitrogen gas blowing to a volume of 1 mL or less. The analytes in the extract are separated by means of capillary column gas chromatography using temperature programming and the phthalate and adipate esters are then measured with a photoionization detector (2-4). Alternatively, a measured volume of sample is extracted with a liquid-solid extraction (LSE) cartridge. The LSE cartridge is eluted with methylene chloride. The eluant is then concentrated by use of a gentle nitrogen purge to a volume of 1 mL or less.

3. DEFINITIONS

3.1 Laboratory reagent blank (LRB) -- An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.2 Field reagent blank (FRB) -- Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.3 Laboratory fortified blank (LFB) -- An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.

3.4 Laboratory fortified sample matrix (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.5 Stock standard solution -- A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.6 Primary dilution standard solution -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.7 Calibration standard (CAL) -- a solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.8 Quality control sample (QCS) -- a sample matrix containing method analytes or a solution of method analytes in a water miscible solvent which is used to fortify reagent water or environmental samples. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance with externally prepared test materials.

4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in water, solvents, reagents, glassware, and sampling processing hardware. These lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks (10.2).

4.1.1 Phthalate esters are contaminants in many products found in the laboratory. It is particularly important to avoid the use of plastics because phthalates are commonly used as plasticizers and are easily extracted from plastic materials. Great care must be exercised to prevent contamination. Exhaustive clean up of reagents and glassware must be required to eliminate background phthalate that is not derived from the sample.

4.1.2 Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry and heat in an oven or muffle furnace at 400°C for 1 hour. Do not heat volumetric glassware. Thorough rinsing with acetone may be substituted for the heating. After cooling, the glassware should be sealed with aluminum foil and stored in a clean environment to prevent accumulation of dust and other contaminants.

4.1.3 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in an all glass system may be required. WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives added by the manufacturer are removed thus potentially reducing the shelf-life.

4.2 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary from source to source, dependent upon the nature and diversity of the industrial complex or municipality being sampled. Clean up procedures can be used to overcome many of these interferences.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified (5-7) for the information of the analyst. (All specifications are suggested, catalog numbers are included for illustration only.)

6. APPARATUS AND MATERIALS

6.1 Sampling Equipment

6.1.1 Grab Sample Bottle--1-L or 1-qt amber glass, fitted with a screw cap lined with teflon. Foil may be substituted for teflon if the sample is not corrosive. Protect samples from light if amber bottles are not available. The bottle and cap liner must be washed, rinsed with acetone or methylene chloride and dried before use in order to minimize contamination. (See 4.1.1.)

6.2 Glassware

6.2.1 Separatory Funnel--2-L with teflon stopcock.

6.2.2 Drying Column--Chromatographic column-300 mm long x 10 mm ID, with teflon stopcock and coarse frit filter disc at bottom (Kontes K - 420540-0213 or equivalent).

6.2.3 Concentrator Tube--Kuderna-Danish, 10 mL, graduated (Kontes K-570050-1025) or equivalent), calibration must be checked at the volumes employed in the test. Tight ground glass stopper is used to prevent evaporation of extracts.

6.2.4 Evaporative Flask--Kuderna-Danish, 500 mL (Kontes K-57000-0500 or equivalent). Attach to concentrator tube with springs.

6.2.5 Snyder Column--Kuderna-Danish, three-ball macro size (Kontes K-503000-0121 or equivalent).

6.2.6 Snyder Column--Kuderna-Danish, 2 or 3 ball micro size (Kontes 590025-0125 or equivalent).

6.2.7 Vials--10 to 15 mL, amber glass with teflon-lined screw cap.

6.2.8 Boiling Chips--Approximately 10/40 mesh. Heat to 400°C for 30 min. or extract with methylene chloride in a Soxhlet apparatus.

6.2.9 Flask, Erlenmeyer--250 mL. (A.H. Thomas Co. #1590-033 or equivalent.)

6.2.10 Chromatography column similar to 6.2.2.

6.2.11 Pasteur Pipets (and Bulb)--VWR Scientific 14672-200 or equivalent.

6.2.12 Autosampler Vials--Equipped with teflon-lined septum and threaded or crimp top caps.

6.3 Water Bath--Heated (with concentric ring covers) capable of temperature control (± 2°C). The water bath should be used in a ventilating hood.

6.4 Balance--Analytical, capable of weighing accurately to nearest 0.0001 gm.

6.5 Gas Chromatograph--An analytical system complete with temperature programmable GC fitted with split-splitless injection mode system, suitable for use with capillary columns and all required accessory syringes, analytical columns, gases, detector and stripchart recorder. A data system for processing chromatographic data is recommended. The gas chromatograph was interfaced with the Nelson Analytical "760" System, IBM personal computer display, personal computer AT, IBM Model M Keyboard and Epson FX-85 select type recorder.

6.5.1 Column, Fused Silica Capillary--DB-5 or equivalent, 30 m long x 0.32 mm ID with a film thickness of 0.25 micron.

6.5.2 The alternate column, Fused Silica Capillary--30 m long x 0.32 mm ID with a film thickness of 0.25 micron, DB-1 or equivalent.

6.5.3 Detector -- A high temperature photoionization detector equipped for 10.0 electron volts and capable of operating from 250°C to 350°C is required. (Tracor Instruments, Inc., Model 703 or equivalent.)

6.5.4 An automatic injector system is suggested, but was not used for the development of this method.

6.6 Vacuum pump, 110 VAC, capable of maintaining a vacuum of 8-10 mm Hg.

7. REAGENTS AND CONSUMABLE MATERIALS

7.1 Reagent Water--Reagent water is defined as water in which an interfering substance is not observed at the MDL of the parameters of interest. Reagent water used to generate data in this method was distilled water obtained from the Millipore L/A-7044 system comprised of prefiltration, organic adsorption, deionization and Millipore filtration columnar units.

7.2 Acetone, hexane, methylene chloride, ethyl acetate, ethyl ether and isooctane -- Pesticide quality or equivalent to distillation in glass quality.

7.3 Sodium Sulfate--(ACS) Granular, anhydrous. Several levels of purification may be required in order to reduce background phthalate levels towards acceptance: 1) Heat 4 h at 400°C in a shallow tray, 2) Soxhlet extract with methylene chloride for 48 h.

7.4 Florisil--PR grade (60/100 mesh). To prepare for use, place 100 g of Florisil into a 500-mL beaker and heat for approximately 16 h at 40°C. After heating transfer to a 500-mL reagent bottle. Tightly seal and cool to room temperature. When cool, add 3 mL of reagent water. Mix thoroughly by shaking or rolling for 10 min. and let it stand for at least 2 h. Store in the dark in glass containers with ground glass stoppers or foil-lined screw caps.

7.5 Sodium Chloride--(ACS) Granular. Heat 4 h at 400°C in a shallow tray. When cool, keep in tightly sealed bottle.

7.6 Ethyl Ether--(ACS) reagent grade.

7.7 Sodium Thiosulfate ($Na_2S_2O_3$)--(ACS) reagent grade.

7.8 Alumina--Neutral activity Super I, W200 series (ICN Life Sciences Group, No. 404583). To prepare for use, place 100 g of alumina into a 500-mL beaker and heat for approximately 16 h at 400°C. After heating transfer to a 500-mL reagent bottle. Tightly seal and cool to room temperature. When cool, add 3 mL of reagent water. Mix thoroughly by shaking or rolling for 10 min. and let it stand for at least 2 h. Keep the bottle sealed tightly.

7.9 Liquid-solid extraction (LSE) cartridges. Cartridges are inert non-leaching plastic, for example polypropylene, or glass, and must not contain plasticizers, such as phthalate esters or adipates, that leach into methylene chloride. The cartridges are packed with about 1 gram of silica, or other inert inorganic support, whose surface is modified by chemically bonded octadecyl (C_{18}) groups. The packing must have a narrow size distribution and must not leach organic compounds into methylene chloride. One liter of water should pass through the cartridge in about 2 hrs with the assistance of a slight vacuum of about 13 cm (5 in.) of mercury. The extraction time should not vary unreasonably among LSE cartridges.

7.10 Helium carrier gas, as contaminant free as possible.

7.11 Stock standard solutions (1.00 µg/µL) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

7.11.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in isooctane and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

7.11.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

7.11.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicates a problem. Butylbenzyl phthalate is especially vulnerable to autoxidation.

7.12 Laboratory control sample concentrate - See Sect. 10.3.1.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Grab samples must be collected in glass containers. Conventional sampling practices should be followed (8,9); however, the bottle must not be prerinsed with sample before collection.

8.2 SAMPLE PRESERVATION AND STORAGE

8.2.1 For sample dechlorination, add 60 mg sodium thiosulfate to the sample bottle at the sampling site or in the laboratory before shipping to the sampling site.

8.2.2 After the sample is collected in a bottle containing preservative(s), seal the bottle and shake vigorously for 1 min.

8.2.3 The samples must be iced or refrigerated at 4°C free from light from the time of collection until extraction. Limited holding studies have indicated that the analytes thus stored are stable up to 14 days or longer. Analyte stability may be affected by the matrix; therefore, the analyst should verify that the preservation technique is applicable to the particular samples under study.

8.3 Extract Storage -- Extracts should be stored at 4°C in absence of light. A 14-day maximum extract storage time is recommended. The analyst should verify appropriate extract holding times applicable to the samples under study.

9. CALIBRATION

9.1 Establish gas chromatograph operating conditions equivalent to those given in Table 1. The gas chromatographic system can be calibrated using the external standard technique (Section 9.2).

9.1.1 Performance of the detector should be checked daily by a specified procedure given in the gas chromatograph operator's manual. If the response is weak, the ultraviolet lamp is removed carefully following disconnection of the power supply. It is cleaned and then placed into its original position with the aid of a leak detector.

9.2 External standard calibration procedure:

9.2.1 Prepare calibration standards at a minimum of three concentration levels for each analyte of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with n-hexane. One of the external standards should be at a concentration near, but above, the MDL (Table 2) and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

9.2.2 Using injection of 1 to 2 µL, analyze each calibration standard according to Sect. 11.4 and tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

10. QUALITY CONTROL

10.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks, and QC samples. Additional quality control practices are recommended.

10.2 Laboratory Reagent Blanks. Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window of any analyte of interest the LRB produces a peak that would prevent the determination of that analyte using a known standard, determine the source of contamination and eliminate the interference before processing samples.

10.3 Initial Demonstration of Capability.

10.3.1 Select a representative spike concentration, about 10 times EDL or at the regulatory Maximum Contaminant Level (MCL), (whichever is lower) for each analyte. Prepare a laboratory control sample concentrate (in methanol) containing each analyte at 1000 times selected concentration. With a syringe, add 1 mL of the concentrate to each of seven 1-L aliquots of reagent water, and analyze each aliquot according to procedures in Sect. 11.1 or 11.2, and 11.3 and 11.4.

10.3.2 For each analyte, the mean recovery value should fall in the range of R ± 30% (or within R ± $3S_r$ if broader) using the values for R and S_r for reagent water in Table 3 or Table 4. For those compounds that meet the acceptance criteria, performance is considered acceptable and sample analysis may begin. For those compounds that fail these criteria, initial demonstration procedures should be repeated.

10.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. It is expected that, as laboratory personnel gain experience with this method, the quality of data will improve beyond those required here.

10.4 The analyst is permitted to modify GC columns, GC detectors, GC conditions, concentration techniques, internal standards or surrogate compounds. Each time such method modifications are made, the analyst must repeat the procedures in Sect. 10.3.

10.5 Assessing Laboratory Performance - Laboratory Fortified Blank

10.5.1 The laboratory must analyze at least one laboratory fortified blank (LFB) sample per sample set (all samples extracted within a 24-h period). The spiking concentration of each analyte in the LFB should be 10 times MDL or the MCL, whichever is less. Calculate accuracy as percent recovery, R. If the recovery of any analyte falls outside the control limits (see Sect. 10.5.2), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

10.5.2 Until sufficient internal data become available, usually a minimum of results from 20 to 30 analyses, the laboratory should assess laboratory performance against the control limits in Sect. 10.3.2 that are derived from the control limits developed during the initial demonstration of capability (10.3). When sufficient internal performance data becomes available, develop control limits from the mean percent recovery, R, and standard deviation, S_r, of the percent recovery. These data are used to establish upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = R + 3S_r$$

$$\text{LOWER CONTROL LIMIT} = R - 3S_r$$

After each five to ten new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points.

10.6 Assessing Analyte Recovery -- Laboratory Fortified Sample Matrix

10.6.1 The laboratory must fortify each analyte to a minimum of 10% of the routine samples or one fortified sample per set, whichever is greater. The fortified concentration should not be less than the background concentration of the sample selected for fortifying. Ideally, this concentration should be the same as that used for the laboratory fortified blank (Sect. 10.5). Over time, samples from all routine sample sources should be fortified.

10.6.2 Calculate the accuracy as percent recovery, R, for each analyte, corrected for background concentrations measured in the unfortified sample, and compare these values to the control limits established in Sect. 10.5.2 from the analyses of LFBs.

10.6.3 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Sect. 10.5), the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. The result for that analyte in the unspiked sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

10.7 QUALITY CONTROL SAMPLES (QCS) - Each quarter, the laboratory should analyze one or more QCS (if available). If criteria provided with the QCS are not met, corrective action should be taken and documented.

10.8 The laboratory may adopt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements.

11. PROCEDURE

11.1 LIQUID-LIQUID EXTRACTION

11.1.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel containing 50 g of NaCl.

11.1.2 Add a mixture of 40 ml CH_2Cl_2 5 mL hexane, and 5 ml ethyl acetate to the sample bottle. Seal, and shake gently 5 seconds to rinse the inner walls of the bottle. Transfer the solvent to the separatory funnel. Extract the sample by shaking the funnel for 2 min with initial and periodic venting to release excess pressure. Allow the organic layer to separate for a minimum of 10 min from the water phase. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the solvent extract in a 250-mL Erlenmeyer flask.

11.1.3 Add a second 50-mL volume of above mixture to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner. Then extract with 40-mL of hexane, which extract (top phase) is added to the total.

11.1.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D concentrator, provided the concentration factor attained in 11.6 - 11.8 is achieved without loss of analytes.

11.1.5 Pour the combined extract through a drying column (6.2.2) containing about 10 cm of prerinsed anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

11.1.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 40 min. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches approximately 7 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

11.1.7 Increase the temperature of the hot water bath to about 85°C. Remove the Snyder column, rinse the column and the 500-mL evaporative flask with 10 - 15 ml of methylene chloride. Replace with a micro column and evaporative flask. Concentrate the extract as in Sect. 11.1.6 to 0.5 - 1 mL. The elapsed time of concentration should be approximately 15 min.

11.1.8 Remove the micro Snyder column and rinse the column by flushing with hexane using a 5-mL syringe. Concentrate to a volume of 1 ml by purging the liquid surface with a gentle flow of nitrogen. Transfer the extract to an autosampler vial with a Pasteur pipet. Seal the vial with a threaded or crimp top cap. Store in refrigerator if further processing will not be performed. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis (Sect. 11.4). If the sample requires further cleanup, proceed to Sect. 11.3.

11.1.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11.2 LIQUID-SOLID EXTRACTION

11.2.1 This method is applicable to a wide range of organic compounds that are efficiently partitioned from the water sample onto a C_{18} organic phase chemically bonded to a solid inorganic matrix, and are sufficiently volatile and thermally stable for gas chromatography (10). Particulate bound organic matter will not be partitioned, and more than trace levels of particulates in the water may disrupt the partitioning process. Single laboratory accuracy and precision data have been determined at a single concentration for the analytes listed in 1.1 fortified into reagent water and raw source water.

11.2.2 Set up the extraction apparatus shown in Figure 1A. The reservoir is not required, but recommended for convenient operation. Water drains from the reservoir through the LSE cartridge and into a syringe needle which is inserted through a rubber stopper into the suction flask. A slight vacuum of 13 cm (5 in.) of mercury is used during all operations with the apparatus. With this extraction apparatus, sample elution requires approximately 2 hours. Acceptable new cartridge and extraction disk technology have recently become available, which allow significantly faster elution rates.

11.2.3 Mark the water meniscus on the side of the sample bottle (approximately 1 liter) for later determination of sample volume. Pour the water sample into the 2-L separatory funnel with the stopcock closed.

11.2.4 Flush each cartridge with two 10 mL aliquots of methylene chloride, followed by two 10 mL aliquots of methanol, letting the cartridge drain dry after each flush. These solvent flushes may be accomplished by adding the solvents directly to the solvent reservoir in Figure 1A. Add 10-mL of reagent water to the solvent reservoir, but before the reagent water level drops below the top edge of the packing in the LSE cartridge, open the stopcock of the separatory funnel and begin adding sample water to the solvent reservoir. Close the stopcock when an adequate amount of sample is in the reservoir.

11.2.5 Periodically open the stopcock and drain a portion of the sample water into the solvent reservoir. The water sample will drain into the cartridge, and from the exit into the suction flask. Maintain the packing material in the cartridge immersed in water at all times. After all of the sample has passed through the LSE cartridge, wash the separatory funnel and cartridge with 10 mL of reagent water, and draw air through the cartridge for 10 min.

11.2.6 Transfer the 125-mL solvent reservoir and LSE cartridge (from Figure 1A) to the elution apparatus (Figure 1B). The same 125 mL solvent reservoir is used for both apparatus. Wash the 2-liter separatory funnel with 5 mL of methylene chloride and collect the washings. Close the stopcock on the 100-mL separatory funnel of the elution apparatus, add the washings to the reservoir and enough additional methylene chloride to bring the volume back up to 5 mL and elute the LSE cartridge. Elute the LSE cartridge with an additional 5 mL of methylene chloride (10-mL total). A small amount of nitrogen positive pressure may be used to elute the cartridge. Small amounts of residual water from the LSE cartridge will form an immiscible layer with the methylene chloride in the 100-mL separatory funnel. Open the stopcock and allow the methylene chloride to pass through the drying column packed with anhydrous sodium sulfate (1-in) and into the collection vial. Do not allow the water layer to enter the drying column. Remove the 100 mL separatory funnel and wash the drying column with 2 mL of methylene chloride. Add this to the extract. Concentrate the extract to 1 mL under a gentle stream of nitrogen. The extract is now ready for gas chromatography (11.4) or additional cleanup (11.3).

11.3 EXTRACT CLEANUP - Cleanup procedures may not be necessary for a relatively clean sample matrix, such as most drinking waters. If particular circumstances demand the use of a cleanup procedure, the analyst may use either procedure below or any other appropriate procedure. However, the analyst first must demonstrate that the requirements of Sect. 10.3 and 10.5 can be met using the method as revised to incorporate the cleanup procedure.

11.3.1 Florisil column cleanup for phthalate esters:

11.3.1.1 Place 10 g of Florisil (see 7.4) into a chromatographic column. Tap the column to settle the Florisil and add 1 cm of anhydrous sodium sulfate to the top.

11.3.1.2 Preelute the column with 40 mL of hexane. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, quantitatively transfer the sample extract (11.1.8 or 11.2.6) onto the column, using an additional 2 mL of hexane to complete the transfer. Just prior to exposure of the sodium sulfate layer to the air, add 40 mL of hexane and continue the elution of the column. Discard this hexane eluate.

11.3.1.3 Next, elute the column with 100 mL of 20% ethyl ether in hexane (V/V) into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Elute the column at a rate of about 2 mL/min for all fractions. Concentrate the collected fraction as in Section 11.1. No solvent exchange is necessary. Adjust the volume of the cleaned extract to 1 mL in the concentrator tube and analyze by gas chromatography.

11.3.2 Alumina column cleanup for phthalate esters:

11.3.2.1 Place 10 g of alumina into a chromatographic column. Tap the column to settle the alumina and add 1 cm of anhydrous sodium sulfate to the top.

11.3.2.2 Preelute the column with 40 mL of hexane. The rate for all elutions should be about 2 mL/min. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, quantitatively transfer the sample extract (11.1.8 or 11.2.6) onto the column, using an additional 2 mL of hexane to complete the transfer. Just prior to exposure of the sodium sulfate layer to the air, add 35 mL of hexane and continue the elution of the column. Discard this hexane eluate.

11.3.2.3 Next, elute the column with 140 mL of 20% ethyl ether in hexane (V/V) into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Concentrate the collected fraction as in Section 11.1. No solvent exchange is necessary. Adjust the volume of the cleaned extract to 1 mL in the concentrator tube and analyze by gas chromatography.

11.4 GAS CHROMATOGRAPHY

11.4.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included are retention data for the primary and confirmation columns. Other capillary (open-tubular) columns, chromatographic conditions, or detectors may be used if the requirements of Section 10 are met.

11.4.2 Calibrate the system daily as described in Sect. 9.

11.4.3 Inject 1 to 2 µL of the sample extract or standard into the gas chromatograph. Smaller (1.0 µL) volumes may be injected if automatic devices are employed. For optimum reproducibility, an autoinjector is recommended.

11.4.4 Identify the analytes in the sample by comparing the retention times of the peaks in the sample chromatogram with those of the peaks in standard chromatograms. The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.4.5 If the response for a peak exceeds the working range of the system, dilute the extract and reanalyze.

11.4.6 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

11.4.7 The calibration curves should be linear over the range of concentrations in Tables 2-5.

12. CALCULATIONS

12.1 Calculate the amount of material injected from the peak response using the calibration curve or calibration factor determined in Section 9.2.2. The concentration in the sample can be calculated from Equation 2.

Equation 2.

$$\text{Concentration } (\mu g/L) = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:
A = Amount of material injected (ng).
V_i = Volume of extract injected (μL).
V_t = Volume of total extract (μL).
V_s = Volume of water extracted (mL).

12.2 Report results in μg/L without correction for recovery data. All QC data obtained should be reported with the sample results.

13. METHOD PERFORMANCE

Single laboratory accuracy and precision data were obtained by replicate liquid-liquid extraction analyses of reagent water fortified at two sets of concentrations of method analytes. The data are given in Tables 2 and 3. Accuracy and precision data by liquid-solid extraction of reagent water fortified at a single concentration are given in Table 4. Finally, method validation data obtained by the analyses of fortified tap water and raw source water are given in Tables 5-7.

14. REFERENCES

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TABLE 1. RETENTION DATA AND CHROMATOGRAPHIC CONDITIONS

Parameter	Retention Time (min) Column 1	Retention Time (min) Column 2
Dimethyl phthalate	17.23	17.89
Diethyl phthalate	20.29	21.13
Di-n-butyl phthalate	27.57	28.67
Butylbenzyl phthalate	34.19	35.34
Bis(2-ethylhexyl) adipate	34.85	36.76
Bis(2-ethylhexyl) phthalate	37.51	39.58
Di-n-octyl phthalate	41.77	44.44

Column 1: DB-5, fused silica capillary, 30 m x 0.32 mm I.D., 0.25 micron film thickness, Helium linear velocity = 30 cm/s.

Column 2: DB-1, fused silica capillary, 30 m x 0.32 mm I.D., 0.25 micron film thickness, Helium linear velocity = 30 cm/s.

Chromatographic Conditions:
Injector temperature = 295°C
Detector temperature = 295°C
Program - 1 min hold at 60°C, 6°C/min to 260°C, 10 min hold.
Splitless injection with 45 s delay

TABLE 2. ACCURACY, PRECISION, AND METHOD DETECTION LIMIT DATA FROM SIX LIQUID-LIQUID EXTRACTION ANALYSES OF FORTIFIED REAGENT WATER

Analyte	True Conc. μg/L	Mean Meas. Conc. μg/L	Std. Dev. μg/L	Mean Accuracy % of True Conc.	MDL μg/L
Dimethyl phthalate	2.02	1.42	0.38	70.3	1.14
Diethyl phthalate	1.51	1.16	0.28	76.8	0.84
Di-n-butyl phthalate	2.62	1.78	0.41	67.9	1.23
Butyl benzyl phthalate	6.00	3.27	0.89	54.5	2.67
Bis(2-ethylhexyl) adipate	6.03	3.94	1.44	65.3	11.82
Bis(2-ethylhexyl) phthalate	5.62	2.92	0.75	52.0	2.25
Di-n-octyl phthalate	17.18	7.96	2.14	46.3	6.42

TABLE 3. ACCURACY AND PRECISION DATA FROM SEVEN LIQUID-LIQUID EXTRACTION ANALYSES OF FORTIFIED REAGENT WATER

Analyte	True Concentration µg/L	Mean Accuracy % of True Concentration	Relative Standard Deviation %
Dimethyl phthalate	15	73	16
Diethyl phthalate	15	71	16
Di-n-butyl phthalate	15	68	15
Butyl benzyl phthalate	15	71	15
Bis(2-ethylhexyl) adipate	30	69	18
Bis(2-ethylhexyl) phthalate	30	67	21
Di-n-octyl phthalate	30	62	23

TABLE 4. ACCURACY AND PRECISION DATA FROM SIX LIQUID-SOLID EXTRACTION ANALYSES OF FORTIFIED REAGENT WATER

Analyte	True Concentration µg/L	Mean Accuracy % of True Concentration	Relative Standard Deviation %
Dimethyl phthalate	15	74	11
Diethyl phthalate	15	85	10
Di-n-butyl phthalate	15	74	11
Butyl benzyl phthalate	15	72	14
Bis(2-ethylhexyl) adipate	30	84	11
Bis(2-ethylhexyl) phthalate	30	101	13
Di-n-octyl phthalate	30	85	13

TABLE 5. ACCURACY AND PRECISION DATA FROM SIX LIQUID-LIQUID EXTRACTION ANALYSES OF FORTIFIED TAP WATER

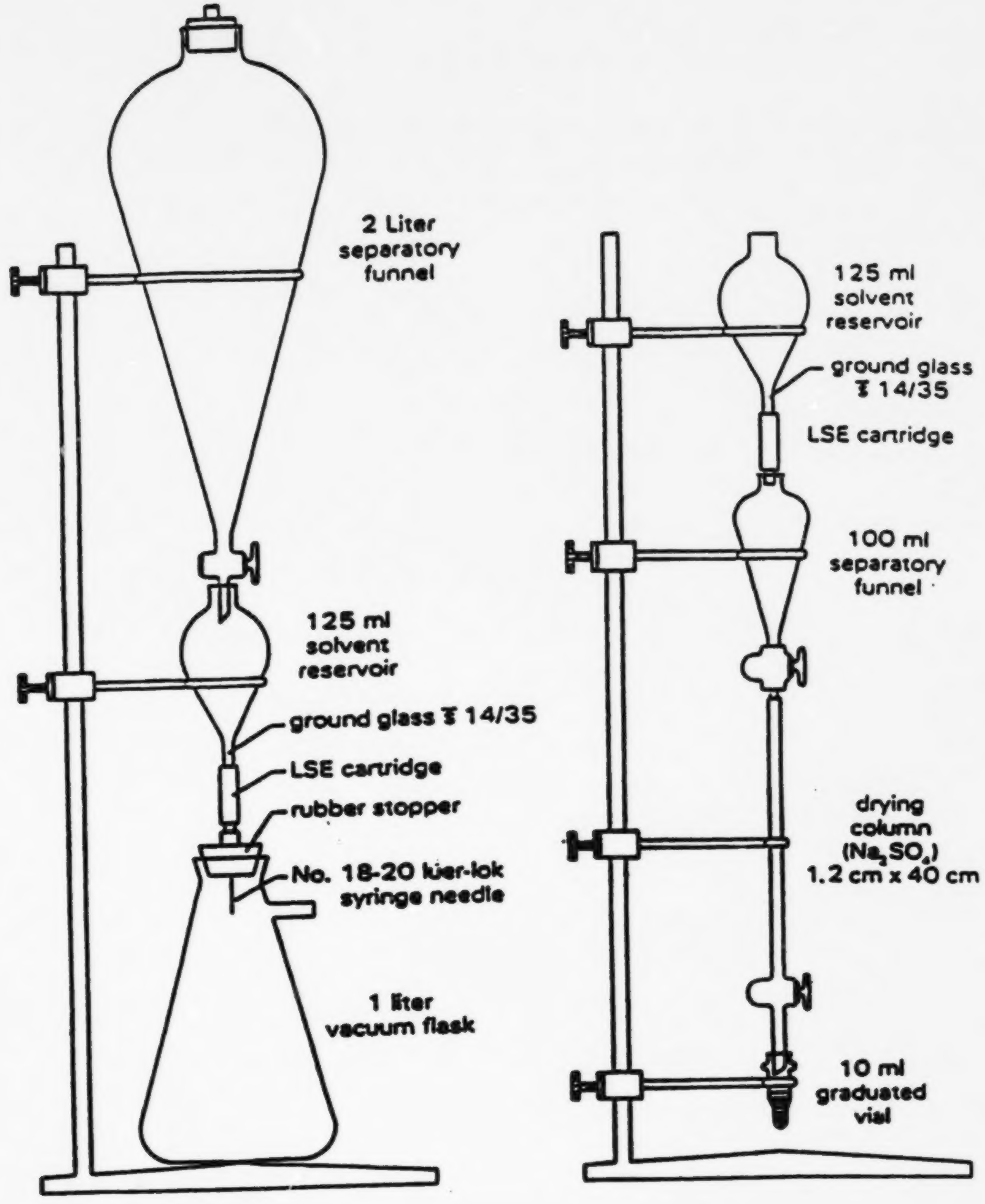
Analyte	True Concentration µg/L	Mean Accuracy % of True Concentration	Relative Standard Deviation %
Dimethyl phthalate	5	103	10.0
Diethyl phthalate	5	106	10.0
Di-n-butyl phthalate	5	94	6.8
Butyl benzyl phthalate	5	93	9.1
Bis(2-ethylhexyl) adipate	5	87	12.0
Bis(2-ethylhexyl) phthalate	5	93	4.9
Di-n-octyl phthalate	5	72	26.0

TABLE 6. ACCURACY AND PRECISION DATA FROM SIX LIQUID-LIQUID EXTRACTION ANALYSES OF FORTIFIED RAW SOURCE WATER

Analyte	True Concentration µg/L	Mean Accuracy % of True Concentration	Relative Standard Deviation %
Dimethyl phthalate	5	59	51
Diethyl phthalate	5	78	45
Di-n-butyl phthalate	5	99	29
Butyl benzyl phthalate	5	72	23
Bis(2-ethylhexyl) adipate	5	115	32
Bis(2-ethylhexyl) phthalate	5	91	35
Di-n-octyl phthalate	5	54	24

TABLE 7. ACCURACY AND PRECISION DATA FROM SIX LIQUID-SOLID EXTRACTION ANALYSES OF FORTIFIED RAW SOURCE WATER

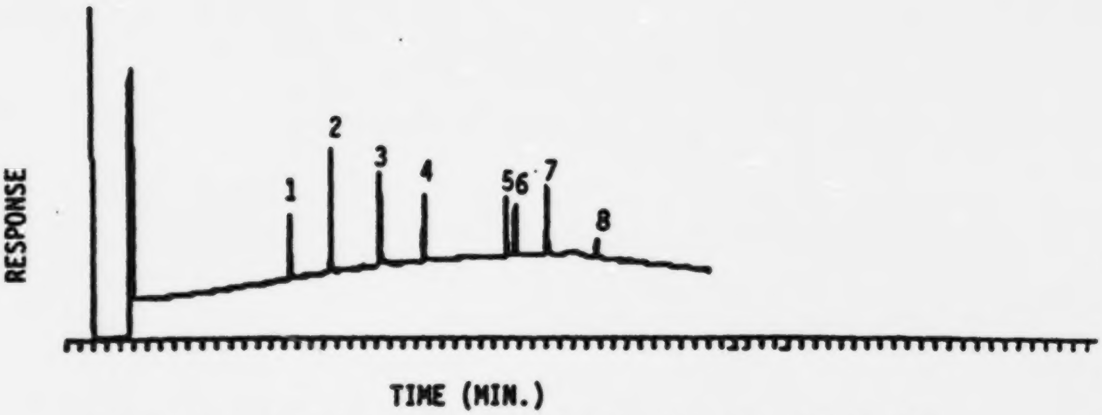
Analyte	True Concentration µg/L	Mean Accuracy % of True Concentration	Relative Standard Deviation %
Dimethyl phthalate	5	110	20
Diethyl phthalate	5	111	32
Di-n-butyl phthalate	5	95	30
Butyl benzyl phthalate	5	82	20
Bis(2-ethylhexyl) adipate	5	65	24
Bis(2-ethylhexyl) phthalate	5	60	21
Di-n-octyl phthalate	5	53	15


2 Liter
separatory
funnel
125 ml
solvent
reservoir
ground glass ⌶ 14/35
LSE cartridge
rubber stopper
No. 18-20 luer-lok
syringe needle
1 liter
vacuum flask
125 ml
solvent
reservoir
ground glass
⌶ 14/35
LSE cartridge
100 ml
separatory
funnel
drying
column
(Na_2SO_4)
1.2 cm x 40 cm
10 ml
graduated
vial

A. Extraction apparatus

B. Elution apparatus

FIGURE 1



Peaks obtained by injecting 5 ng for the 1st, 2nd, 4th and 5th compounds, 10 ng for the 6th, 7th and 8th compounds, and 2.5 ng for the 3rd compound. (Table 1)

FIGURE 2

METHOD 513. DETERMINATION OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN DRINKING WATER BY GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY

July 1990

This method is taken from the SW-846 Methods Manual,
Method 8280, and adapted to drinking water.

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METHOD 513

DETERMINATION OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN DRINKING WATER BY GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY

1. SCOPE AND APPLICATION

1.1 This method provides procedures for identification and measurement of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, CASRN 1746-01-6) at concentrations of 20 pg/L to 2 ng/L in water sample extracts. The minimum measurable concentration will vary among samples, depending on the presence or absence of interfering compounds in a particular sample.

1.2 A water sample may contain floating, suspended, and settled particulate matter, which should not be removed by filtering before extraction. The estimated solubility of 2,3,7,8-TCDD in water is <50 ng/L (1), but larger measured concentrations can be caused by TCDD associated with particulates.

1.3 Because 2,3,7,8-TCDD may be extremely toxic, safety procedures described in Section 5 should be followed to prevent exposure of laboratory personnel to materials containing this compound.

2. SUMMARY OF METHOD

2.1 An entire 1-L water sample is transferred to a separatory funnel, and two isotopically-labeled analyte analogs, $^{37}Cl_4$-2,3,7,8-TCDD (surrogate compound, SC) and $^{13}C_{12}$-2,3,7,8-TCDD (internal standard, IS), are added to the water. The sample container is rinsed with methylene chloride, which is then added to the water sample. The water sample is extracted sequentially with three 60-mL portions of methylene chloride. The combined extract is subjected to column chromatographic procedures to remove sample components that may interfere with detection and measurement of TCDD. A 10-µL aliquot of a solution containing $^{13}C_{12}$-1,2,3,4-TCDD, which is used as a recovery standard (RS), is added to the extract before concentration and analysis. The sample extract is concentrated to 10 µL, and a 1-µL or 2-µL aliquot is injected into a gas chromatograph (GC) equipped with a fused silica capillary column and interfaced with a high resolution mass spectrometer (MS). Selected characteristic ions are monitored with high resolution MS (10,000 resolving power). Identification of a sample component as TCDD is based on detection of two characteristic ions (m/z 320 and 322) in the molecular ion cluster, measurement of acceptable relative abundance of those two ions, and determination of the retention time of the candidate sample component relative to the IS, $^{13}C_{12}$-2,3,7,8-TCDD. Because the IS is a labeled analog of the analyte, the procedure presumes that IS losses during method procedures are equal to unlabeled TCDD losses. Therefore, each calculated sample TCDD concentration has been compensated for losses during sample preparation.

3. DEFINITIONS

3.1 Calibration limits -- the minimum (20 pg/L) and maximum (2 ng/L) concentration of 2,3,7,8-TCDD in solutions used to calibrate detector response. In some samples, <20 pg/L of 2,3,7,8-TCDD may be detected but measured concentrations will only be estimated concentrations. In other samples, interferences may prevent identification and measurement of 20 pg/L.

3.2 Concentration calibration solution -- a solution containing known amounts of the analyte (unlabeled 2,3,7,8-TCDD), the IS ($^{13}C_{12}$-2,3,7,8-TCDD), the SC ($^{37}Cl_4$-2,3,7,8-TCDD), and the RS ($^{13}C_{12}$-1,2,3,4-TCDD); it is used to determine instrument response to the analyte, SC, and RS relative to response to the IS.

3.3 Field blank -- a portion of reagent water that has been shipped to the sampling site and exposed to conditions that samples have experienced.

3.4 Internal standard (IS) -- $^{13}C_{12}$-2,3,7,8-TCDD, which is added to every sample and is present at the same concentration in every blank, quality control sample, and concentration calibration solution. It is added to the water sample before extraction and is used to measure the concentration of unlabeled TCDD.

3.5 Laboratory reagent blank -- a blank prepared in the laboratory by performing all analytical procedures except a 1-L aliquot of reagent water is extracted rather than a sample.

3.6 Performance check solution -- a solution containing a mixture of known amounts of selected standard compound; it is used to demonstrate continued acceptable GC/MS system performance.

3.7 Recovery standard (RS) -- A compound ($^{13}C_{12}$-1,2,3,4-TCDD) that is present in every calibration solution and is added to each extract just before analysis. It is used to measure the recovery of the internal standard.

3.8 Response factor (RF) -- response of the mass spectrometer to a known amount of analyte relative to a known amount of internal standard.

3.9 Signal-to-noise ratio (S/N) -- the ratio of the area of the analyte signal to the area of the random background signal; it is determined by integrating the signal for a characteristic ion in a region of the selected ion current profile where only random noise is observed and relating that area to the area measured for a positive response for the same ion. The same number of scans must be integrated for both areas. (The ratio of peak heights may be used instead of peak areas.)

3.10 Surrogate compound (SC) -- a compound ($^{37}Cl_4$-2,3,7,8-TCDD) that is present in each calibration solution and is added at a low concentration (20 pg/L) to each sample and blank before extraction. Successful detection and measurement of the SC in each sample provides some assurance that unlabeled 2,3,7,8-TCDD would be detectable if present in the sample at $\geq$20 pg/L.

4. INTERFERENCES

4.1 An organic compound that has approximately the same GC retention time 2,3,7,8-TCDD (within a few scans of the IS) and produces the ions that are monitored to detect 2,3,7,8-TCDD is a potential interference. Most frequently encountered interferences are other sample components that are extracted along with TCDD; some potential interferences are listed in Table 1. To minimize interference, high purity reagents and solvents must be used and all equipment must be meticulously cleaned. Laboratory reagent blanks must be analyzed to demonstrate lack of contamination that would interfere with 2,3,7,8-TCDD measurement. Column chromatographic procedures are used to remove some sample components; these procedures must be performed carefully to minimize loss of 2,3,7,8-TCDD during attempts to enrich its concentration relative to other sample components.

4.2 False positive identifications are produced only when an interfering compound elutes from the GC column within 3 sec of the IS and produces ions with exact masses and relative abundances very similar to those for 2,3,7,8-TCDD. The specified GC column separates 2,3,7,8-TCDD from all 21 other TCDD isomers.

5. SAFETY

5.1 Because 2,3,7,8-TCDD has been identified as an animal carcinogen and a possible human carcinogen, exposure to this compound and its isotopically labeled analogs must be minimized (2,3). The laboratory is responsible for maintaining a file of current OSHA regulations regarding the safe handling of chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in analyses.

5.2 Each laboratory must develop a strict safety program for handling 2,3,7,8-TCDD. The following laboratory practices are recommended:

5.2.1 Minimize laboratory contamination by conducting all manipulations in a hood.

5.2.2 Effluents of GC sample splitters and GC/MS vacuum pumps should pass through either a column of activated carbon or be bubbled through a trap containing oil or high-boiling alcohols.

5.2.3 Liquid waste should be dissolved in methanol or ethanol and irradiated with ultraviolet light at a wavelength <290 nm for several days. Analyze the liquid wastes and dispose of the solutions when 2,3,7,8-TCDD can no longer be detected.

5.3 The following precautions for safe handling of 2,3,7,8-TCDD in the laboratory are presented as guidelines only. These precautions are necessarily general in nature, because detailed specific recommendations can be made only for the particular exposure and circumstances of each individual use. Assistance in evaluating the health hazards of particular conditions may be obtained from certain consulting laboratories or from state Departments of Health or of Labor, many of which have an industrial health service. Although 2,3,7,8-TCDD is extremely toxic to certain kinds of laboratory animals, it has been handled for years without injury in analytical and biological laboratories. Techniques used to handle radioactive and infectious materials are applicable to 2,3,7,8-TCDD.

5.3.1 Protective equipment: Laboratory hood adequate for radioactive work, safety glasses, and disposable plastic gloves, apron or lab coat.

5.3.2 Training: Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.3 Person hygiene: Thorough washing of hands and forearms after each manipulation and before breaks (coffee, lunch, and shift).

5.3.4 Confinement: Isolated work area, posted with signs, segregated glassware and tools, plastic-backed absorbent paper on benchtops.

5.3.5 Waste: Good technique includes minimizing contaminated waste. Plastic liners should be used in waste cans.

5.3.6 Disposal of Wastes: Refer to the November 7, 1986, issue of the Federal Register on Land Ban Rulings for details concerning handling wastes containing dioxin.

5.3.7 Decontamination: Personnel - any mild soap with plenty of scrubbing action. Glassware, tools, and surfaces - rinse with 1,1,1-trichloroethane, then wash with any detergent and water. Dish water may be disposed to the sewer after percolation through a carbon filter. Solvent wastes should be minimized, because they require special disposal through commercial sources that are expensive.

5.3.8 Laundry: Clothing known to be contaminated should be disposed with the precautions described under "Disposal of Hazardous Wastes". Laboratory coats or other clothing worn in 2,3,7,8-TCDD work area may be laundered. Clothing should be collected in plastic bags. Persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. The clothing may be put into a washer without contact if the launderer knows the problem. The washer should be run through one full cycle before being used again for other clothing.

5.3.9 Wipe tests: A useful method to determine cleanliness of work surfaces and tools is to wipe a surface area of 2 in. X 1 ft. with an acetone-saturated laboratory wiper held in a pair of clean stainless steel forceps. Combine wipers to make one composite sample in an extraction jar containing 200 mL acetone. Place an equal number of unused wipers in 200 mL acetone and use as a control. Extract each jar with a wrist-action shaker for 20 min. Transfer extract to a Kuderna-Danish (K-D) apparatus fitted with a concentrator tube and a three-ball Snyder column. Add two boiling chips and concentrate the extract to an apparent volume of 1.0 mL with the same techniques used for sample extracts. Add 100 µL of the sample fortification solution that has not been diluted with acetone or 1.5 mL of the acetone-diluted solution (Section 7.14), and continue all extract preparation steps and analytical procedures described for samples. If any 2,3,7,8-TCDD is detected, report the result as a quantity (picograms) per wipe test. A lower limit of calibration of 20 pg/composite wipe test is expected. A positive response for the control sample is 8 pg/wipe test. When the sample contains $\geq$25 pg, steps must be taken to correct the contamination. First vacuum the working places (hoods, benches, sink) using a vacuum cleaner equipped with a high-efficiency particulate absorbent filter and then wash with a detergent. Analyze a new set of wipers before personnel are allowed in work in the previously contaminated area.

5.3.10 Inhalation: Any procedure that may produce airborne contamination must be carried out with good ventilation. Gross losses to a ventilation system must not be allowed. Handling the dilute solutions normally used in analytical and animal work presents no significant inhalation hazards except in case of an accident.

5.3.11 Accidents: Remove contaminated clothing immediately, taking precautions not to contaminate skin or other articles. Wash exposed skin vigorously and repeatedly until medical attention is obtained.

6. APPARATUS AND EQUIPMENT

6.1 Computerized GC/MS System

6.1.1 The GC must be capable of temperature programming and be equipped with all required accessories, such as syringes, gases, and capillary columns. The GC injection port must be designed for capillary columns. Splitless or on-column injection technique is recommended. With this method, a 2-µL injection is used consistently. A 1-µL injection volume can be used, but the injection volume should be constant throughout analyses of calibration solutions and related blanks, sample extracts, and quality control samples.

6.1.2 GC/MS interface components should withstand temperatures up to 280°C. The interface should be designed so that separation of 2,3,7,8-TCDD from all other TCDD isomers achieved in the GC column is not appreciably degraded. Cold spots or active surfaces (adsorption sites) in the interface can cause peak tailing and broadening. The GC column should be inserted directly into the MS ion source without being exposed to the ionizing electron beam. Graphite ferrules should be avoided in the injection port because they may adsorb TCDD. Vespel or equivalent ferrules are recommended.

6.1.3 The static resolving power of the MS must be maintained at $\geq$10,000 (10% valley). The MS must be operated in a selected ion monitoring (SIM) mode, and data must be acquired for the ions listed in Table 2 during a total cycle time (including instrument overhead time) of $\leq$1 s. Selection of the lock-mass ion is left to the performing laboratory. Recommended MS tuning conditions are provided in Section 9.1. The ADC zero setting must allow peak-to-peak measurement of baseline noise for every monitored channel and allow good estimation of instrument resolving power.

6.1.4 A dedicated data system is used to control rapid SIM data collection. Quantitation data (peak areas or peak heights) must be acquired continuously and stored. The data system must be capable of producing selected ion current profiles (SICPs, which are displays of ion intensities as a function of time) for each monitored ion, including the lock-mass ion. Quantitation may be based on computer-generated peak areas or on measured peak heights. The data system must be capable of acquiring data for $\geq$ five ions and generating hard copies of SICPs for selected GC retention time intervals and permit measurement of baseline noise.

6.2 GC Column. Two narrow bore, fused silica capillary columns coated with phenyl cyanopropyl silicone are recommended; one is a 60-m SP-2330 and the other is a 50-m CP-SIL 88. Any capillary column that separates 2,3,7,8-TCDD from all other TCDD isomers may be used, but this separation must be demonstrated. At the beginning of each 12-h period during which analyses are to be performed, column operating conditions must be demonstrated to achieve the required separation on the column to be used for samples. Operating conditions known to produce acceptable results with the recommended columns are shown in Table 3.

6.3 Miscellaneous Equipment.

6.3.1 Nitrogen evaporation apparatus with variable flow rate.

6.3.2 Balances capable of accurately weighing to 0.01 g and 0.0001 g.

6.3.3 Centrifuge.

6.3.4 Water bath equipped with concentric ring covers and capable of being temperature controlled within ±2°C.

6.3.5 Glove box.

6.3.6 Drying oven.

6.3.7 Minivials -- 1-mL amber borosilicate glass with conical-shaped reservoir and screw caps lined with Teflon-faced silicone disks.

6.3.8 Pipets, disposable, Pasteur, 150 mm X 5 mm i.d.

6.3.9 Separatory funnels, 2 L with Teflon stopcock.

6.3.10 Kuderna-Danish concentrator, 500 mL, fitted with 10-mL concentrator tube and three-ball Snyder column.

6.3.11 Teflon boiling chips washed with hexane before use.

6.3.12 Chromatography column, glass, 300 m X 10.5 mm i.d., fitted with Teflon stopcock.

6.3.13 Adapters for concentrator tubes.

6.3.14 Continuous liquid-liquid extractor (optional).

6.3.15 Glass funnels, appropriate size to accommodate filter paper used to filter extract (volume of approximately 170 mL).

6.3.16 Desiccator.

6.4 CAUTION: To avoid the risk of using contaminated glassware, all glassware that is reused must be meticulously cleaned as soon as possible after use. Rinse glassware with the last solvent used in it. Wash with hot water containing detergent. Rinse with copious amounts of tap water and several portions of distilled. Drain dry and heat in a muffle furnace at 400°C for 15-30 min. Volumetric glassware must not be heated in a muffle furnace. Some thermally stable materials (such as PCBs) may not be removed by heating in a muffle furnace. In these cases, rinsing with high-purity acetone and hexane may be substituted for muffle-furnace heating. After the glassware is dry and cool, rinse it with hexane and store it inverted or capped with solvent-rinsed aluminum foil in a clean environment.

6.5 TCDD concentrations of concern in water are much lower than those of concern in many other sample types. Extreme care must be taken to prevent cross-contamination between water and other samples. The use of separate glassware for water samples is recommended

7. REAGENTS AND CONSUMABLE MATERIALS.

7.1 Alumina, acidic (BioRad Lab. #132-1240 or equivalent). Extract in a Soxhlet apparatus with methylene chloride for 6 h ($\geq$ 3 cycles/h) and activate it by heating in a foil-covered glass container for 24 h at 190°C.

7.2 Carbon, (Amoco PX-21 or equivalent).

7.3 Glass wool. Extract with methylene chloride and hexane and air-dry before use. Store in a clean glass jar.

7.4 Potassium hydroxide, ACS grade.

7.5 Potassium silicate. Slowly dissolve 56 g of reagent grade potassium hydroxide in 300 mL of anhydrous methanol in a 1-L round bottom flask. Add slowly with swirling 100 g silica gel (prewashed and activated). With a rotary evaporation apparatus with no vacuum applied, rotate the flask and heat to 55°C for 90 min. After the mixture cools to room temperature, pour it into a large glass column containing a plug of glass wool at the end. Wash the mixture into the column with methanol, and then add 200 mL of methanol. When the methanol level reaches the bed of sorbent, add 200 mL of methylene chloride to the column. Push the methylene chloride through the column of sorbent by applying nitrogen pressure to dry or partially dry the sorbent, which is then activated at 130°C overnight.

7.6 Silica gel, high purity grade, type 60, 70-230 mesh. Extract in a Soxhlet apparatus with methylene chloride for 6 h ($\geq$3 cycles/h) and activate by heating in a foil-covered glass container for 24 h at 130°C.

7.7 Silica gel impregnated with 40% (w/w) sulfuric acid. Add two parts (by weight) concentrated sulfuric acid to three parts (by weight) silica gel (extracted and activated), mix with a glass rod until free of lumps, and store in a screw-capped glass bottle.

7.8 Silica gel/Carbon. To a 20-g portion of silica gel add 500 mg carbon, and blend until the mixture is a uniform color.

7.9 Sodium sulfate, granular, anhydrous.

7.10 Solvents, high purity, distilled-in-glass, or highest available purity: methylene chloride, hexane, benzene, methanol, tridecane, isooctane, toluene, cyclohexane, and acetone.

7.11 Sulfuric acid, concentrated, ACS grade, specific gravity 1.84.

7.12 Concentration Calibration Solutions (Table 4) -- Five (or more) tridecane solutions (CAL 1-5) containing unlabeled 2,3,7,8-TCDD and isotopically labeled TCDDs. All five solutions contain unlabeled 2,3,7,8-TCDD at varying concentrations and the IS ($^{13}C_{12}$-2,3,7,8-TCDD, CASRN 80494-19-5) and the RS ($^{13}C_{12}$-1,2,3,4-TCDD) each at a constant concentration. Three of these solutions also contain the surrogate compound (SC, $^{37}Cl_4$-2,3,7,8-TCDD, CASRN 85508-50-5) at varying concentrations. All standards required for preparing CALs are commercially available but must be verified by comparison with the National Bureau of Standards certified solution SRM-1614, which contains 67.8 ng/mL of unlabeled 2,3,7,8-TCDD and 65.9 ng/mL of ^{13}C-labeled 2,3,7,8-TCDD at 23°C. Note: CALs can be prepared by diluting calibration solutions used in Contract Laboratory Program procedures for 2,3,7,8-TCDD determinations with low resolution MS; to obtain appropriate IS concentrations for CAL 4, however, solvent containing the IS must be used for dilution. Calibration solutions used for USEPA Method 8290 can also be used to determine RFs for 2,3,7,8-TCDD; with those solutions the lower calibration concentration may be higher (25 pg/L rather than 20 pg/L) or lower, depending on injected volume of calibration solution. Because Method 8290 solutions do not contain the SC, however, one or three additional solutions containing that compound will be necessary to measure its RF relative to the IS. Assuming adequate reproducibility of RF measurements, triplicate analyses of one solution (recommended SC concentration of 1.2 pg/μL) or single analysis of three solutions (0..6 to 1.8 pg/μL, Table 4) are acceptable.

7.12.1 Each of CALs 1-5 contains the IS at a concentration of 50 pg/μL; if 100% of the IS is extracted, 10 μL of this solution is equivalent to a 10-μL extract of a 1-L sample to which 500 pg of IS was added before extraction. If 100% of the analyte is extracted, CALs 1-5 contain unlabeled 2,3,7,8-TCDD at concentrations that are equivalent to 10-μL extracts of 1-L samples containing 20 pg to 2 ng.

7.12.2 CALs 1-3 contain the SC (^{37}Cl-2,3,7,8-TCDD) at a concentration of 0.6 pg/μL, 1.2 pg/μL, and 1.8 pg/μL, respectively; 10 μL of those solutions are equivalent to 10 μL extracts containing 30%, 60%, and 90%, respectively, of the amount of SC added to each 1-L sample before extraction.

7.12.3 Store CALS in 1-mL amber minivials at room temperature in the dark.

7.13 Column Performance Check Solution -- contains a mixture of TCDDs including the IS, the SC, unlabeled 2,3,7,8-TCDD, 1,2,3,4-TCDD (CASRN 30746-58-8), 1,4,7,8-TCDD(CASRN 40581-94-0), 1,2,3,7,-TCDD (CASRN 67028-18-6), 1,2,3,8-TCDD (CASRN 53555-02-5), 1,2,7,8-TCDD (CASRN 34816-53-0), and 1,2,6,7-TCDD (CASRN 40581-90-6). Other TCDDs can be present. Except for the IS and SC, solution component concentrations are not critical. The IS concentration should be 10 ± 1 pg/µL and the SC concentration should be 0.6 ± 0.1 pg/L, because ions produced by these compounds will be used to check signal-to-noise ratios.

7.14 Sample Fortification Solution. A solution containing the IS at a concentration of 5 to 25 pg/µL and the SC at a concentration of 0.2 to 1 pg/µL, but with the ratio of IS to SC always 25:1. The solution solvent is not critical; mix 20 to 100 µL, as appropriate to produce needed IS and SC concentrations (50 pg/L and 2 pg/L, respectively) of this solution with 1.5 mL of acetone. Add the resulting solution to each sample and blank before extraction.

7.15 Recovery Standard Solution. A tridecane solution containing the recovery standard, $^{13}C_{12}$-1,2,3,4-TCDD at a concentration of 50 pg/µL. A 10-µL aliquot of this solution is added to each sample and blank extract before concentrating the extract to its final volume for analysis.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE.

8.1 Samples must be collected in glass containers. The container should not be rinsed with sample before collection.

8.2 Samples may be stored under ambient conditions as long as temperature extremes (below freezing or >90°F) are avoided. To prevent photo-decomposition, samples must be protected from light from the time of collection until extraction.

8.3 All samples must be extracted within 90 days after collection and completely analyzed within 40 days after extraction. Extracts must be held for 6 months before they are disposed.

9. GC/MS SYSTEM CALIBRATION.

9.1 MS Performance.

9.1.1 The MS must be operated in the electron ionization mode, and a static resolving power of ≥10,000 (10% valley definition) at ≥m/z 334 must be demonstrated before any analysis is performed. The resolving power must be documented by recording the mass profile of the reference peak. The format of the peak profile representation must allow manual determination of resolution (i.e., the horizontal axis must be a calibrated mass scale (amu or ppm per division). The peak width at 5% peak height must appear on the hard copy and cannot exceed 100 ppm. Static resolving power must be checked at the beginning and end of each 12-h period of operation. A visual check of static resolution (by using the peak matching unit) is recommended before and after each analysis.

9.1.2 Chromatography time may exceed the long-term mass stability of the high resolution MS, and mass drift of a few ppm can affect the accuracy of measured masses. Therefore, a mass drift correction is required. A lock mass ion from the reference compound (high boiling perfluorokerosene, PFK, is recommended) is used to calibrate the MS. An acceptable lock mass is an ion with mass larger than the lightest mass monitored but less than the heaviest ion monitored. The amount of PFK introduced into the ion chamber during analysis should be adjusted so that the amplitude of the lock mass ion is <10% full scale. Excessive PFK may cause noise problems and ion source contamination.

9.1.3 Using a PFK molecular leak, tune the MS to obtain resolving power of $\geq$10,000 (10% valley) at m/z 334. With a peak matching unit and a reference peak near m/z 320, verify that the exact mass of the reference peak is within 5 ppm of the known mass. The low- and high-mass reference ions must be selected to provide the voltage jump required to detect ions from m/z 320 through m/z 334. (Note: With a qualitative confirmation option in Section 11.4.5, detected ion range will be m/z 257 to m/z 334.)

9.1.4 MS resolving power must be demonstrated by recording the mass peak profile of the high-mass reference signal obtained during peak matching by using the low-mass ion as a reference. The minimum resolving power of 10,000 must be demonstrated on the high-mass ion while it is transmitted at a lower accelerating voltage than the low-mass reference ion, which is transmitted at full sensitivity. The peak profile representation must allow manual determination of the resolution (i.e., the horizontal axis must be a calibrated mass scale in amu or ppm per division. The measured peak width at 5% of the peak maximum must appear on the hard copy and cannot exceed 100 ppm at the high mass.

9.2 Initial Calibration.

9.2.1 GC column performance. The laboratory must verify GC conditions necessary for required separation of 2,3,7,8-TCDD from other TCDD isomers. Inject 2 µL of the performance check solution and acquire SIM data for the five ions in Table 2 (nominal m/z 320, 322, 328, 332, and 334) within a total cycle time of $\leq$1 s. Acquire at least five scans for each ion across each GC peak and use the same data acquisition time for each ion monitored. The peak representing 2,3,7,8-TCDD and peaks representing any other TCDD isomers must be resolved with a valley of $\leq$25% (Figure 1), Valley % = 100 x/y, where y is peak height of 2,3,7,8-TCDD and x is measured as shown in Figure 1 between 2,3,7,8-TCDD and its closest eluting isomer. CAUTION: The same data acquisition parameters must be used to analyze all calibration and performance check solutions.

9.2.2 MS calibration and sensitivity check. Ratio of integrated ion current for m/z 320 to m/z 322 produced by unlabeled 2,3,7,8-TCDD and for m/z 332 and 334 produced by the IS (^{13}C-labeled 2,3,7,8-TCDD) must be $\geq$0.67 and $\leq$0.87. The S/N ratio for m/z 328 produced by the SC (^{13}C-labeled 1,2,3,4-TCDD) must be $\geq$2.5 and the S/N ratio for m/z 332 produced by the IS must be $\geq$10.

9.2.3 Using the same GC and MS conditions, analyze a 2-μL aliquot of the medium concentration CAL (CAL 3). Check ion ratios specified in Section 9.2.2. If criteria are met, analyze a 2-μL aliquot of each of the four (or more) remaining CALS.

9.2.4 For each CAL, ensure that ion ratios (Section 9.2.2) are acceptable. For CAL 1 (the lowest concentration CAL) data, ensure that each ion produces a signal-to-noise (S/N) ratio of >2.5. Display a SICP for a region of the chromatogram near the elution time of 2,3,7,8-TCDD but where no analyte or interference peak is present. The preferred width of the display is about 10 X full width at half height of the IS peak. The "noise" is the height (measured from the lowest point in the display window) of the largest signal not attributable to any eluting substance.

9.2.5 RF Measurements. Using data acquired for each CAL, calculate the RF for unlabeled 2,3,7,8-TCDD, the SC ($^{37}Cl_4$-2,3,7,8-TCDD), and the RS ($^{13}C_{12}$-2,3,7,8-TCDD) relative to the IS ($^{13}C_{12}$-2,3,7,8-TCDD) with the following equation:

$$RF = A_x Q_{is} / A_{is} Q_x$$

where A_x = the sum of integrated ion abundances of m/z 320 and 322 for unlabeled 2,3,7,8-TCDD, the abundance of m/z 328 for the SC, or the abundances of m/z 332 and 334 for the RS.

A_{is} = the sum of integrated ion abundances of m/z 332 and 334 for the IS,

Q_{is} = injected quantity of IS, and

Q_x = injected quantity of unlabeled 2,3,7,8-TCDD, the SC, or the RS.

RF is a unitless number; units used to express quantities must be equivalent.

9.2.6 For each compound (unlabeled 2,3,7,8-TCDD, the SC, and the RS), calculate a mean RF and the relative standard deviation (RSD) of the five measured RFs. When RSD exceeds 20%, analyze additional aliquots of appropriate CALs to obtain an acceptable RSD of RFs over the entire concentration range, or take action to improve GC/MS performance.

9.3 Routine Calibration. If a laboratory operates during only one ≤12-h period (shift) each day, routine calibration procedures must be performed at the beginning (after mass calibration and successful analysis of the performance check solution to ensure adequate sensitivity and acceptable ion ratios) of that shift, and the performance check solution must be analyzed again at the end of that shift to validate data acquired during the shift. If the laboratory operates during consecutive shifts, routine calibration procedures must be performed at the beginning of each shift, but analysis of the performance check solution at the beginning of each shift and at the end of the final 12-h period is sufficient.

9.3.1 Inject a 2-µL aliquot of CAL 3, and analyze with the same conditions used during Initial Calibration.

9.3.2 Demonstrate acceptable performance for ions abundance ratios, and demonstrate that each measured RF for unlabeled 2,3,7,8-TCDD, the SC, and the RS is within 20% of the appropriate mean RF measured during initial calibration. If one or more of these criteria are not met, up to two additional attempts can be made before remedial action is necessary and the entire initial calibration process is repeated. Corrective action may include increasing the detector sensitivity, baking the GC column, clipping a short length (about 0.3-0.5 m) of the injector side of the GC column, washing or replacing the GC column, and cleaning the ion source. If degradation of the standards in CALs is suspected, a fresh set of CALs should be used for repeating initial calibration procedures.

10. QUALITY CONTROL.

10.1 Laboratory Reagent Blank. Perform all steps in the analytical procedure using all reagents, standards, equipment, apparatus, glassware, and solvents that would be used for a sample analysis, but omit a water sample, and substitute 1 L of reagent water.

10.1.1 Analyze two laboratory reagent blanks (LRBs) before sample analyses begin and when a new batch of solvents or reagents is used for sample extraction. Do not add any IS, SC or RS to one blank; this will allow demonstration that reagents contain no impurities producing any ion current above the level of background noise for monitored ions.

10.1.2 Criteria for acceptable LRBs.

10.1.2.1 When no IS, SC, or RS is present, no ion current above the level of background S/N is detected for any monitored ion within 20 s of the retention times previously measured for labeled 1,2,3,4-TCDD or for unlabeled and labeled 2,3,7,8-TCDD.

10.1.2.2 When the IS is present, no ion current for m/z 259, 320, or 322 is observed that is >2% of the abundance of m/z 332 within 5 scans of the IS peak maximum.

10.1.3 Corrective action for unacceptable LRB. Check solvents, reagents, apparatus, and glassware to locate and eliminate the source of contamination before any samples are extracted and analyzed. Purify or discard contaminated reagents and solvents.

10.2 Field Blanks. An acceptable field blank must meet criteria in Section 10.1.2.2. When results for a field blank are acceptable, analysis of an LRB is not needed with that sample batch. When field blank results are not acceptable, analysis of an LRB is needed; if LRB results are acceptable, data for samples associated with the field blank must be accompanied by pertinent information about the nature and amount of contamination observed in the field blank.

10.3 Corrective action for unacceptable performance check solution data. When the MS sensitivity requirement (Section 9.2.2) is not met at the end of a 12-h period in which sample extracts were analyzed, all related sample extracts must be reanalyzed after criteria have been met. When other criteria (ion ratios or GC resolution) are not met, all sample extracts that produced positive results or potential positive results must be reanalyzed after calibration criteria have been met.

11. PROCEDURE

11.1 Sample Extraction

11.1.1 Mark the water meniscus on the side of the 1-L sample bottle for later determination of the exact sample volume. Pour the entire sample (approximately 1 L) into a 2-L separatory funnel. A continuous liquid-liquid extractor may be used instead of a separatory funnel.

11.1.2 Add 1.5 mL of the sample fortification solution (Section 7.14) to the sample in the separatory funnel.

11.1.3 Add 60 mL of methylene chloride to the sample bottle, seal and shake 30 s to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting. Allow the organic layer to separate from the water phase for a minimum of 10 min. If an emulsion interface between layers exists, the analyst may use mechanical techniques to complete the phase separation. Collect the methylene chloride layer directly into a 500-mL Kuderna Danish (K-D) concentrator (mounted with a 10-mL concentrator tube) by passing the sample extract through a filter funnel packed with a glass wool plug and 5-g of anhydrous sodium sulfate. Repeat the extraction with two additional 60-mL portions of methylene chloride, filtering each extract before adding it to the K-D concentrator. After the third extraction, rinse the sodium sulfate with an additional 30 mL of methylene chloride to ensure quantitative transfer, and add rinse to composite extract.

11.1.4 Add one or two clean boiling chips to the evaporative flask and attach a Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (60-65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Concentrate the extract until the apparent volume of the liquid reaches 1 mL. Remove the K-D apparatus and allow it to drain and cool for at least 10 min. Remove the Snyder column, add 50 mL of hexane and a new boiling chip and reattach the Snyder column. Increase the water bath temperature to 85-90°C and concentrate the extract to approximately 1 mL. Rinse the flask and the lower joint with 1-2 mL hexane. Concentrate the extract to 1 mL under a gentle stream of nitrogen. If further extract processing is to be delayed, the extract should be quantitatively transferred to a Teflon-sealed, amber, screw-cap vial and stored refrigerated and protected from light.

11.1.5 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11.2 Sample Extract Cleanup

11.2.1 Chromatography columns 1 and 2, described below, are recommended for every sample extract. A third column containing silica gel and carbon may be useful for removal of interferences from some sample extracts and may be used at the analyst's discretion. Because each cleanup procedure increases the chances of analyte loss, such procedures should be minimized. Criteria for predicting when the carbon column will be needed are not available, but that column is probably not needed for finished drinking water samples that have been filtered through granular activated carbon.

11.2.2 Column Preparation

11.2.2.1 Column 1. Place 1.0 g of silica gel (See NOTE) into a 1.0 cm X 20 cm column and tap the column gently to settle the silica gel. Add 2 g potassium hydroxide impregnated silica gel, 1 g silica gel, 4.0 g of sulfuric acid impregnated silica gel, and 2 g silica gel. Tap column gently after each addition. NOTE: The silica gel for this application is partially deactivated with 1% water immediately before packing the column.

11.2.2.2 Column 2. Place 6.0 g of alumina into a 1.0 cm X 30 cm column and tap the column gently to settle the alumina. Add a 1-cm layer of purified sodium sulfate to the top of the alumina.

11.2.2.3 Add hexane to each column until the packing is free of channels and air bubbles. A small positive pressure (5 psi) of clean nitrogen can be used if needed.

11.2.3 Quantitatively transfer the sample extract to the top of the silica gel in column 1. Rinse the concentrator tube with two 0.5 mL portions of hexane; transfer rinses to Column 1. With 90 mL of hexane, elute the extract from Column 1 directly into Column 2.

11.2.4 Add an additional 20 mL of hexane to Column 2 and elute until the hexane level is just below the top of the sodium sulfate; discard the eluted hexane.

11.2.5 Add 20 ml of 20% methylene chloride/80% hexane (v/v) to Column 2 and collect the eluate.

11.2.6 If carbon column cleanup is selected, proceed with Section 11.2.7. If not, proceed with Section 11.2.8.

11.2.7 Optional cleanup with Column 3. Reduce the volume of eluate from Column 2 to about 1 mL in a K-D apparatus. Transfer the concentrated eluate from Column 2 to a 4 mm X 200 mm column (2 mL disposable pipette) containing 200 mg silica gel/carbon. Elute with 15 mL methylene chloride and 15 mL 80% methylene chloride/20% benzene (v/v) in forward direction of flow. Discard these fractions. Elute TCDD with 15 mL toluene in a reverse direction flow. Collect this eluate.

11.2.8 Concentrate the eluate (either the toluene fraction from Section 11.2.7 or the methylene chloride/hexane fraction from Section 11.2.5) to a small volume (<0.5 mL) and transfer to a 1-mL minivial. Store the extract in the dark at 4°C until just before analysis. Note: The final volume is adjusted to 10 µL immediately before GC/MS analysis.

11.3 GC/MS Analysis of Extracts

11.3.1 Remove the sample or blank extract from storage and allow it to warm to ambient laboratory temperature. Add a 10-µL aliquot of the RS solution (Section 7.15) to the extract and reduce the extract volume to 10 µL with a stream of dry, purified nitrogen.

11.3.2 Inject a 2-µL aliquot of the extract into the GC, operated under conditions previously used to produce acceptable results with the performance check solution.

11.3.3 Acquire SIM data using the same analytical conditions previously used to determine RFs.

11.4 Identification Criteria

11.4.1 Obtain SICPs for each ion monitored.

11.4.2 The abundance of m/z 332 relative to m/z 334 produced by the IS must be ≥ 0.67 and ≤ 0.87, and these ions must maximize within 1 scan of each other. Retention time should be within ± 5 scans of that observed during the most recent acceptable calibration. For good performance, the retention time of the IS must be reproducible to ± 5 scans from one injection to the next. Over the course of a 12-h work period, the IS retention time should be reproducible within ± 10 scans. Less reproducible IS retention times indicate serious chromatography problems that should be corrected before further sample analyses.

11.4.3 The sample component must produce a signal for both ions monitored to detect and measure unlabeled 2,3,7,8-TCDD, and the abundance of m/z 320 relative to m/z 322 must be ≥ 0.67 and ≤ 0.87. All ions must maximize within 1 scan of each other and within 3 sec of the IS.

11.4.4 The S/N ratio for each unlabeled TCDD and SC ion must be >2.5 and must not have saturated the detector; the S/N ratio for each IS and RS ion must be ≥ 10 and must not have saturated the detector.

11.4.5 Additional qualitative confirmation can be obtained by monitoring m/z 257 and 259 (fragment ions produced by loss of COCl from the analyte) along with ions previously specified or by reanalysis of an aliquot of the extract to monitor m/z 257 and 259 along with m/z 268 and 270, fragment ions produced by loss of COCl from the IS. The relative abundance of m/z 257 to 259 and m/z 268 to 270 should be 0.9 to 1.1, and the abundance of 259 to 270 should be the same as the ratio of 322 to 334 measured in the previous injection. Although variable with instrumental conditions, the abundance of fragment ions relative to molecular ions is approximately 30-45% for each compound; therefore, the detection limit for these ions will be greater than for molecular ions.

12. CALCULATIONS

12.1 From appropriate SICPs of nominal m/z 259, 320 and 322, obtain and record the spectrum number of the apex of the chromatographic peak produced by unlabeled TCDD and the area of the entire chromatographic peak.

12.2 Calculate the concentration using the formula:

$$C_x = (A_x \cdot Q_{is})/(A_{is} \cdot RF \cdot V)$$

where C_x = concentration (picograms per liter),

A_x = sum of areas for m/z 320 and m/z 322 produced by the sample component,

A_{is} = sum of areas for m/z 332 and m/z 334 produced by the IS,

Q_{is} = quantity (picograms) of IS added to the sample,

RF = mean RF measured for unlabeled 2,3,7,8-TCDD during initial calibration, and

V = Volume (liters) of water extracted.

12.3 When fortified samples of known composition are analyzed, calculate the percent method bias using the equation:

$$B = 100\ (C_s - C_t)/\ C_t$$

where C_s = measured concentration (in picograms per liter),

C_t = theoretical concentration (i.e., the concentration resulting from fortification plus any concentration measured in the sample when an unfortified sample extract was analyzed).

NOTE: The bias value retains a positive or negative sign.

12.4 Calculate the IS concentration using the formula:

$$C_{is} = (A_{is} \cdot RF . Q_{rs})/(A_{rs} \cdot V)$$

where C_{is} = concentration (picograms per liter),

A_{is} = sum of areas for nominal m/z 332 and 334 produced by the IS,

A_{rs} = sum of areas for m/z 332 and m/z 334 produced by the RS,

Q_{rs} = quantity (picograms) of RS added to the sample,

RF = mean RF measured for the RS relative to the IS during initial calibration, and

V = Volume (liters) of water extracted.

12.5 Report calculated concentrations with three significant figures when measured concentration is >100 pg/L and with two significant figure when value is <100 pg/L. The recovery of the IS should be $\geq$40% and $\leq$120%.

12.6 Estimated Maximum Possible Concentration (EMPC) -- For samples in which no unlabeled 2,3,7,8-TCDD is detected, calculate the EMPC, which is the concentration required to produce a signal with S/N ratio of 2.5. The background signal level (area or height) within $\pm$5 scans of the IS peak is determined as previously described and is multiplied by 2.5. With the following formula, the product is related to the estimated unlabeled TCDD concentration required to produce a signal equivalent of 2.5 S/N.

$$EMPC = 2.5 \cdot B_x \cdot Q_{is} / A_{is} \cdot RF \cdot V$$

B_x = background (height or area) for either nominal m/z 320 or 322 within $\pm$5 scans of the IS peak,

A_{is} = peak height or area (depending on selection for B_x) for nominal m/z 332 when m/z 320 is used to determine B_x or nominal m/z 334 when m/z 322 is used to determine B_x, and

Q_{is}, RF, and V retain previous definitions.

12.7 An interference results when sample a component elutes in the retention time window for 2,3,7,8-TCDD and produces both monitored TCDD ions but measured relative abundances do not meet identification criteria. Any ion with S/N of <2.5 should be ignored. Ions producing S/N of >2.5 but with unacceptable relative abundance should be treated as an interference, and a quantitative estimate of that interference should be calculated using the equation in Section 12.2. Interferences observed in blanks and also present in samples should not be reported as a sample interference but should be reported as a blank interference.

13. REFERENCES

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3. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Ed., 1979.

4. Statement of Work, Dixoin Analysis, Soil/Sediment and Water Matrices, IFB WA86-K357, September 1986.

TABLE 1. POTENTIAL INTERFERENCES

Compound	Interfering Ion		Required Resolution
	Formula	m/z	
Heptachlorobiphenyl	$M^+ - 2\ ^{35}Cl$	321.867	12,476
Nonachlorobiphenyl	$M^+ - 4\ ^{35}Cl$	319.8521	7,189
	$M^+ - 3\ 3^{5}Cl^{37}Cl$	321.8491	7,233
Tetrachloromethoxy-biphenyl	M^+	319.9329	8,805
	M^+	321.9299	8,848
Tetrachlorobenzyl-phenyl ether	M^+	319.9329	8,813
	M^+	321.9300	8,843
DDT	$M+ - H^{35}Cl$	319.9321	9,006
	$M^+ - H^{35}Cl$	321.9292	9,050
DDE	M^+	319.9321	9,011
	M^+	321.9292	9,050
Pentachlorobenzyl-phenyl ether	$M^+ - H^{35}Cl$	319.9143	18,043
	$M^+ - H^{35}Cl$	321.9114	18,104
Tetrachloroxanthene	M^+	319.9143	18,043
	M^+	321.9114	18,104
Hydroxytetrachloro-dibenzofuran	M^+	319.8966	-
	M^+	321.8936	-
Tetrachlorophenyl-benzoquinone	M^+	319.8966	-
	M^+	321.8936	-

TABLE 2. IONS TO BE MONITORED

Accurate Mass	Elemental Composition	Compound
258.9298	$C_{11}H_4{}^{35}Cl_2{}^{37}ClO$	Unlabeled 2,3,7,8-TCDD
319.8965	$C_{12}H_4{}^{35}Cl_4O_2$	Unlabeled 2,3,7,8-TCDD
321.8936	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	Unlabeled 2,3,7,8-TCDD
327.8847	$C_{12}H_4{}^{37}Cl_4O_2$	$^{37}Cl_4$-2,3,7,8-TCDD (SC)
331.9368 and	$^{13}C_{12}H_4{}^{35}Cl_4O_2$	$^{13}C_{12}$-2,3,7,8-TCDD (IS)
		$^{13}C_{12}$-1,2,3,4-TCDD (RS)
333.9339 and	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	$^{13}C_{12}$-2,3,7,8-TCDD (IS)
		$^{13}C_{12}$-1,2,3,4-TCDD (RS)

TABLE 3. GC OPERATING CONDITION GUIDELINES

Column coating	SP-2330	CP-SIL 88
Film thickness	0.2 um	0.22 um
Column dimensions	60 m X 0.24 mm	50 m X 0.22 mm
Helium* linear velocity	28-29 cm/sec at 240°C	28-29 cm/sec at 240°C
Initial temperature	70°C	45°C
Initial time	4 min	3 min
Temperature program	Rapid increase to 200°C; 200°C to 240°C at 4°C/min	Rapid increase to 190°C; 190°C to 240°C at 5°C/min
Retention time of 2,3,7,8-TCDD	24 min	26 min

*Hydrogen is an acceptable carrier gas.

TABLE 4. COMPOSITION OF CONCENTRATION CALIBRATION SOLUTIONS

	Analyte	Surrogate Cmpd.	Internal Std.	Recovery Std.
CAL #	Unlabeled 2,3,7,8-TCDD	$^{37}Cl_4$-2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD
1	2 pg/µL	0.6 pg/µL	50 pg/µL	30 pg/µL
2	10	1.2	50	30
3	50	1.8	50	30
4	100	0	50	30
5	200	0	50	30

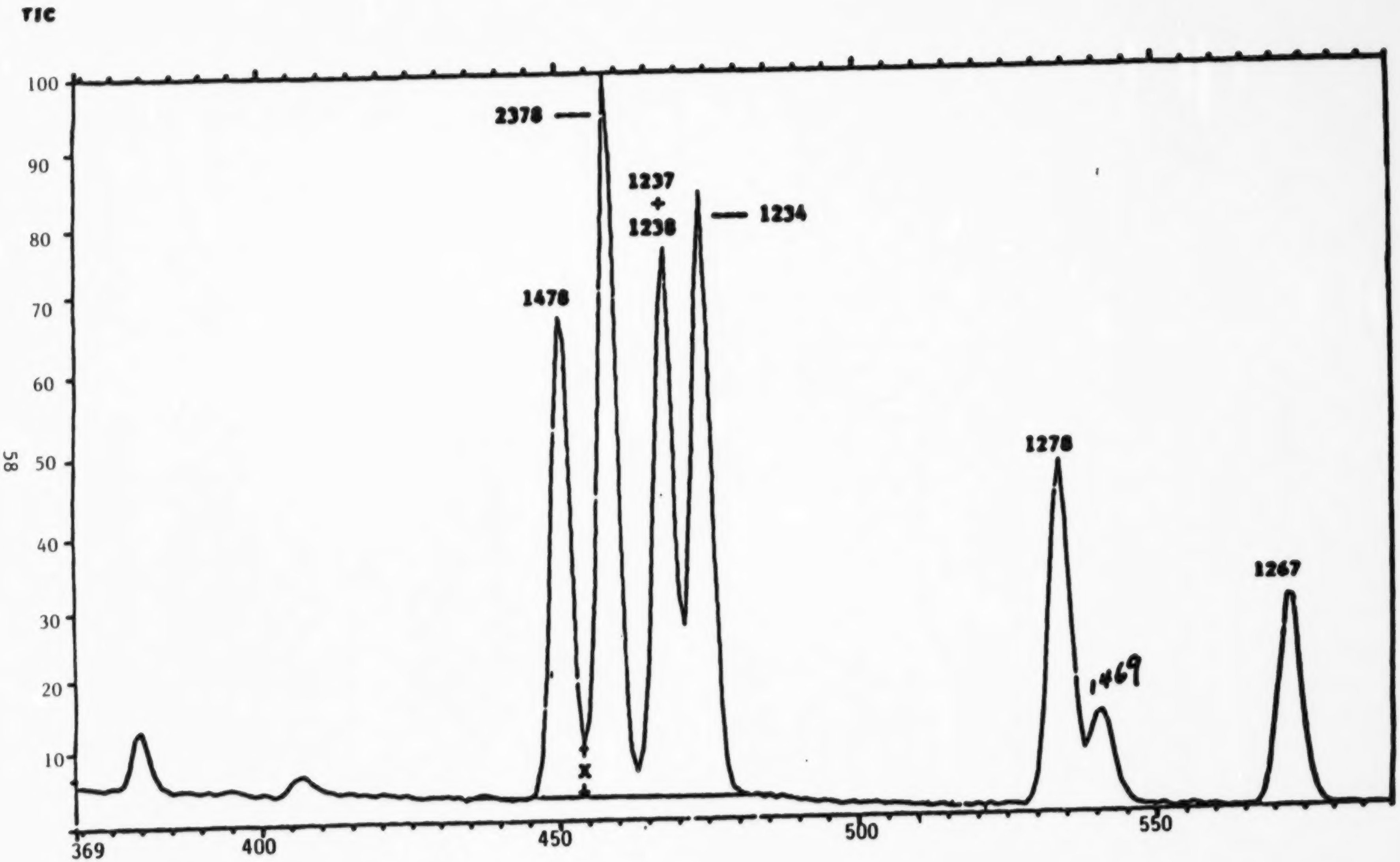

Figure 1. Ion current profile for m/z 210 and 322 produced by analysis of performance check solution using a 50-m CP-SIL 88 fused silica capillary column and conditions listed in Table 3. Percent relative

METHOD 547. ANALYSIS OF GLYPHOSATE IN DRINKING WATER BY DIRECT AQUEOUS INJECTION HPLC WITH POST-COLUMN DERIVATIZATION

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METHOD 547

ANALYSIS OF GLYPHOSATE IN DRINKING WATER BY DIRECT AQUEOUS INJECTION HPLC WITH POST-COLUMN DERIVATIZATION

1. SCOPE AND APPLICATION

1.1 This method describes a procedure for the identification and measurement of Glyphosate (N-phosphonomethyl glycine) in drinking water matrices. Single laboratory accuracy and precision data have been determined for this method.

1.2 Glyphosate was found to rapidly decompose in chlorinated waters (1). It is therefore unlikely that the analyte will be evidenced in tap water except as separate glycine and N-phosphonomethyl moieties, neither of which is applicable to this method.

Analyte	Chemistry Abstract Services Registry Number
Glyphosate	1071-83-6

1.3 The method detection limits (MDL, defined in Section 13) for glyphosate are listed in Table 1 (2). The MDLs for a specific sample may differ from those listed.

2. SUMMARY OF METHOD

2.1 A water sample is filtered and a 200 µL aliquot injected into a cation exchange HPLC column. Separation is achieved by using an isocratic elution. After elution from the analytical column at 65 C, the analyte is oxidized with calcium hypochlorite and the product (glycine) coupled with o-phthaladehyde-2-mercaptoethanol complex at 38 C to give a fluorophor detected by a fluorometer with excitation at 340 nm and detection of emission measured > 455 nm (1,3).

3. DEFINITIONS

3.1 LABORATORY DUPLICATES (LD1 and LD2) - Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.2 FIELD DUPLICATES (FD1 and FD2) - Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.3 LABORATORY REAGENT BLANK (LRB) - An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.4 FIELD REAGENT BLANK (FRB) - Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.5 LABORATORY PERFORMANCE CHECK SOLUTION (LPC) - A solution of method analytes, surrogate compounds, and internal standards used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

3.6 LABORATORY FORTIFIED BLANK (LFB) - An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the method is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.

3.7 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) - An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.8 STOCK STANDARD SOLUTION - A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.9 CALIBRATION STANDARD (CAL) - A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.10 QUALITY CONTROL SAMPLE (QCS) - A sample matrix containing method analytes or a solution of method analytes in a water miscible solvent which is used to fortify reagent water or environmental samples. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance with externally prepared test materials.

4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks as required by Section 10.2.

4.1.1 Glassware must be scrupulously cleaned (4). Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. Glassware should then be drained dry, and heated in a laboratory oven at 400°C for several hours before use. After drying and cooling, glassware should be stored in a clean environment to prevent any accumulation of dust or other contaminants.

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required to achieve necessary purity.

4.2 Samples may become contaminated during shipment or storage. Field blanks must be analyzed to determine that sampling and storage procedures have prevented contamination.

4.3 The extent of matrix interferences may vary considerably from source to source, depending upon the nature and diversity of the matrix being sampled. No interferences have been observed in the matrices studied.

4.4 The extent of interferences that may be encountered using liquid chromatographic techniques has not been fully assessed. Although the HPLC conditions described allow for a unique resolution of the compound covered in this method, other matrix components may interfere.

5. SAFETY

5.1 The toxicity or carcinogenicity of chemicals used in this method has not been precisely defined. Each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals used in this method (5). A reference file of material data handling sheets should be made available to all personnel involved in the chemical analysis.

6. **APPARATUS AND EQUIPMENT** (All specifications are suggested. Catalog numbers are included for illustration only).

6.1 SAMPLING EQUIPMENT (for discrete or composite sampling).

6.1.1 Grab sample bottle - 60 mL screw cap bottles (Pierce No. 13075 or equivalent) and caps equipped with a PTFE-faced silicone septa (Pierce No. 12722 or equivalent). Prior to use, wash vials and septa as described in Section 4.1.1.

6.2 GLASSWARE

6.2.1 Autosampler vials - glass, 3.7 mL, with PTFE-lined septa and screw caps. (Supelco, #2-3219, or equivalent)

6.2.2 Volumetric flask - 1000 mL and 100 mL

6.3 BALANCE - analytical, capable of accurately weighing 0.0001 g.

6.4 pH METER - capable of measuring pH to 0.01 units.

6.5 FILTRATION APPARATUS

6.5.1 Macrofiltration - to filter mobile phase and derivatization solutions used in HPLC system. Membrane filter, 0.2 µ mesh, 47 mm diameter, Nylon 66 (Alltech, #2034 or equivalent)

6.5.2 Microfiltration - to filter samples prior to HPLC analysis. Use 0.45 µ filters (Gelman Acrodisc - CR or equivalent)

6.5.3 Helium, for degassing solutions and solvents.

6.6 SYRINGES

6.6.1 One 250 µL glass syringe, with blunt tip needle for manual injection.

6.6.2 3 - 5 mL disposable hypodermic syringes with Luer-Lok tip.

6.6.3 Micro syringes, various sizes.

6.7 INSTRUMENTATION - A schematic diagram of the analytical system is shown in Figure 1.

6.7.1 A high performance liquid chromatograph (HPLC) capable of injecting 200 µL aliquots and utilizing an isocratic pumping system with constant flow rate of 0.5 mL/min.

6.7.2 Column - 250 x 4 mm, Bio-Rad, Aminex A-9. Column specifications: K^+ form, packed at 65°C, pH = 1.9. This column was used to generate the method performance statements in Section 13. Different HPLC columns may be used if requirements described in Section 10.3 are met. Use of guard columns is recommended.

6.7.3 Guard Column - C_{18} packing - (Dupont, Zorbax Guard Column or equivalent).

6.7.4 Column Oven, (Fiatron, Model CH-30 and controller, Model TC-50, or equivalent).

6.7.5 Post Column Reactor (PCR) - Capable of mixing reagents into the mobile phase. Reactor to be equipped with pumps to deliver 0.5 mL/min of each reagent; mixing tees; two 1.0 mL delay coils, thermostated at 38°C; and constructed using PTFE tubing. (Kratos Model URS 051 and URA 200 or equivalent).

6.7.6 Fluorescence Detector - Capable of excitation at 340 nm and detecting of emission > 455 nm. A Schoeffel Model 970 fluorescence detector was used to generate the validation data presented in this method.

6.7.7 Data System - A strip chart recording of the detector response must be provided as a minimum requirement. The use of a data system to calculate retention times and peak areas is recommended but not required. The system used to generate the data in Table 1 is as follows.

6.7.7.1 IBM AT computer with 640 KB of RAM, a 20 MB hard disk, and enhanced graphics monitor or equivalent.

6.7.7.2 Nelson Analytical Interface and Software Model 2600, Version 4.1 or equivalent to perform data computations.

6.7.7.3 Printer, Epson FX-286 or equivalent for report generation.

7. REAGENTS AND CONSUMABLE MATERIALS

7.1 HPLC MOBILE PHASE

7.1.1 REAGENT WATER - reagent water is defined as water of very high purity, equivalent to distilled-in-glass solvents.

7.1.2 MOBILE PHASE - 0.005 M KH_2PO_4 (0.68 gm) in 960 mL reagent water, add 40 mL HPLC grade methanol, adjust pH of solution to 1.9 with concentrated phosphoric acid then filter with 0.22 μ filter and degas with helium before use.

7.2 POST COLUMN DERIVATIZATION SOLUTIONS

7.2.1 Calcium hypochlorite solution - Dissolve 1.36 g KH_2PO_4, 11.6 g NaCl and 0.4 g NaOH in 500 mL deionized water. Add 15 mg $Ca(ClO)_2$ dissolved in 50 mL deionized water and dilute solution to 1000 mL with deionized water. Filter solution through 0.22 μ membrane filter and degas with helium before use.

7.2.2 O-phthalaldehyde (OPA) reaction solution

7.2.2.1 2-Mercaptoethanol (1+1) - Mix 10.0 mL of 2-mercaptoethanol and 10.0 mL of acetonitrile. Cap store in hood. (Caution - stench)

7.2.2.2 Sodium borate (0.05N) - Dissolve 19.1 g of sodium borate ($Na_2B_4O_7 \cdot 10\ H_2O$) in 1.0 L of reagent water. The sodium borate will completely dissolve at room temperature if prepared a day before use.

7.2.2.3 OPA Reaction Solution - Dissolve 100 ± 10 mg of o-phthalaldehyde (mp 55-58°C) in 10 mL of methanol. Add to 1.0 L of 0.05 N sodium borate. Mix, filter through 0.45 μ membrane filter, and degas. Add 100 μL of 2-mercaptoethanol (1+1) and mix. Make up fresh solution daily unless the reagent solution is protected from atmospheric oxygen. The solution can be stored in glass bottles under atmospheric conditions at 4 C for up to two weeks without appreciable increases in background fluorescence or stored under nitrogen for indefinite periods.

Note: Fluoraldehyde (Pierce Chemical), a commercially formulated OPA reaction solution, may be substituted for Steps 7.2.2.1 through 7.2.2.3.

7.3 SAMPLE PRESERVATION REAGENTS

7.3.1 Sodium thiosulfate - granular - ACS grade or better (Fisher, S-446).

7.4 STOCK STANDARD SOLUTION (1.00 μg/mL)

7.4.1 Accurately weigh and dissolve 0.1000 g of pure glyphosate in 1000 mL of deionized water. Larger or smaller volumes may be used at the convenience of the analyst. If compound purity is certified at 96% of greater, the weight may be used without correction to calculate the concentration of the stock standard.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Collect samples in glass containers (6.1.1). Conventional sampling practices (6) are to be followed.

8.2 SAMPLE PRESERVATION - Treatment of samples to remove residual chlorine will eliminate the possibility of glyphosate losses due to chlorine during storage. Chlorine is destroyed by adding 100 mg/L of sodium thiosulfate to the sample.

8.3 SAMPLE STORAGE - Samples should be stored at 4°C away from light and analyzed within 2 weeks. A preservation study (7) has demonstrated the stability of glyphosate in frozen samples for up to 18 months. The analyst should verify appropriate sample holding times applicable to the sample under study.

9. CALIBRATION

9.1 Establish liquid chromatographic operating conditions indicated in Table 1.

9.2 Prepare a minimum of three calibration standards of glyphosate by serial dilution of the stock standard solution in deionized water. One of the calibration standards should correspond to a glyphosate concentration near to, but above the MDL. The other concentrations should comprise the range of concentrations expected for the samples, or, otherwise, define the working range of the detector.

9.3 Analyze each calibration standard and tabulate peak area against concentration (in µg/L) injected. The results may be used to prepare a calibration curve for glyphosate.

Alternatively, if the ratio of response to concentration (response factor) is constant over the working range (< 10% relative standard deviation), linearity through the origin can be assumed and the average ratio or response factor can be used in place of a calibration curve.

9.4 The working calibration curve must be verified on each working day by the measurement of a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (greater than 8 hr), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analyses. If the response for the analyte varies from the predicted response by more than ± 20%, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve.

10. QUALITY CONTROL

10.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified matrix samples, laboratory fortified blanks and QC samples.

10.2 LABORATORY REAGENT BLANKS. Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, a laboratory reagent blank (LRB) must be analyzed. If within the retention time window of the analyte of interest the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

10.3 INITIAL DEMONSTRATION OF CAPABILITY

10.3.1 Prepare laboratory fortified blanks (LFBs) at an analyte concentration of 250 µg/L. With a syringe, add .250 mL of the stock standard (Section 7.4) to at least four - 100 mL aliquots of reagent water and analyze each aliquot according to procedures beginning in Section 11.

10.3.2 The glyphosate recovery (R) values determined in 10.3.1 should be within ± 30% of the R values listed in Table 2 for at least three of four consecutive samples. The relative standard deviation (Sr) of the mean recovery (R) should be less than 30%. If the analyte of interest meets the acceptance criterion, performance is judged acceptable and sample analysis may begin. For analytes that fail this criterion, initial demonstration procedures should be repeated.

10.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. It is expected that as laboratory personnel gain experience with this method the quality of the data will improve beyond the requirements stated in Section 10.3.2.

10.4 The analyst is permitted to modify HPLC column, HPLC conditions, or detectors to improve separations or lower analytical costs. Each time such method modifications are made, the analyst must repeat the procedures in Section 10.3.

10.5 LABORATORY FORTIFIED BLANKS

10.5.1 The laboratory must analyze at least one laboratory fortified blank (LFB) sample per sample set (all samples analyzed within a 24-h period). The fortified concentration of glyphosate in the LFB should be 10 times the MDL. Calculate accuracy as percent recovery (R). If R falls outside the control limits (See Section 10.5.2.), the analysis is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.

10.5.2 Until sufficient data become available from within their own laboratory, usually a minimum of results from 20 to 30 analyses, the laboratory should assess laboratory performance against the control limits in Section 10.3.2. When sufficient internal performance data become available, develop control limits from the mean percent recovery (R) and S_R of the percent recovery. These data are used to establish upper and lower control limits as follows:

UPPER CONTROL LIMIT = $R + 3S_R$
LOWER CONTROL LIMIT = $X - 3S_R$

After each five to ten new recovery measurements, new control limits should be calculated using only the most recent 20 - 30 data points.

10.6 LABORATORY FORTIFIED SAMPLE MATRIX

10.6.1 The laboratory must add a known fortified concentration to a minimum of 10% of the routine samples or one fortified sample per set, whichever is greater. The fortified concentration should not be less then the background concentration of the original sample. Ideally, the fortified concentration should be the same as that used for the laboratory fortified blank (Section 10.5). Over time, samples from all routine samples sources should be fortified.

10.6.2 Calculate the accuracy as R for the analyte, corrected for background concentrations measured in the original sample, and compare these values to the control limits established in Section 10.5.2 from the analyses of LFBs.

10.6.3 If the recovery of any sample falls outside the designated range, and the laboratory performance for the analyte is shown to be in control (Section 10.5), the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. The result for the analyte in the original sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

10.7 QUALITY CONTROL SAMPLES (QCS) - Each quarter the laboratory should analyze at least one QCS (if available). If criteria provided with the QCS are not met, corrective action should be taken and documented.

10.8 The laboratory may adopt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

11. PROCEDURE

11.1 SAMPLE CLEANUP - The cleanup procedure for this direct aqueous injection HPLC method is limited to the filtration procedure described in Section 11.2.3. Applying only filtration, no interferences were evidenced in the analysis of tap water, ground water and municipal effluent. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must demonstrate that the recovery of the analyte is within limits specified by the method.

11.2 ANALYSIS

11.2.1 Table 1 details the recommended HPLC-PCR operating conditions. An example of the chromatography achieved under these conditions is shown in Figure 2.

11.2.2 Calibrate the system daily as described in Section 9.

11.2.3 Filter samples using 0.45 µ Acrodisc filters (6.5.2) and inject 200 µL of sample into the HPLC-PCR system for analysis.

11.2.4 Record resulting peak sizes in area units.

11.2.5 If the response for a glyphosate peak in a sample chromatogram exceeds the working calibration range, dilute the sample with reagent water and reanalyze.

11.3 IDENTIFICATION OF ANALYTES

11.3.1 Identify a sample component by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds, within limits (11.3.2), to the retention time of the standard, then identification is considered positive.

11.3.2 The width of the retention time window used to make identification should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation in retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.3.3 Identification requires expert judgement when sample components are not resolved chromatographically. When peaks obviously represent more then one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima), or any time doubt exists over the identification of a peak in a chromatogram, appropriate confirmatory techniques such as use of an alternative detector which operates on a physical/chemical principle different from that originally used, e.g., mass spectrometry, or the use of an alternative separation technology, e.g., anion exchange chromatography, must be employed.

12. CALCULATIONS

12.1 Determine the concentration (C) of glyphosate in the sample by direct comparison with the calibration curve described in Section 9, or alternatively, by means of the equation below derived from the calibration data.

$$C\ (\mu g/L) = \frac{A}{RF}$$

where:

A = Area of glyphosate peak in sample
RF = Response factor derived from calibration data

12.2 For samples processed as part of a set where laboratory fortified blank and/or laboratory fortified matrix recoveries fall outside control limits in Section 10.5 & 10.6, data for the affected samples must be labeled as suspect.

13. METHOD PERFORMANCE

13.1 METHOD DETECTION LIMITS - The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above the background level (2). The concentrations listed in Table 1 were obtained using reagent water, ground water and dechlorinated tap water.

13.2 Single-laboratory precision and accuracy results at several concentrations in drinking water matrices are presented in Table 2.

14. REFERENCES

1. Bashe, W. J., T. V. Baker, "Analysis of Glyphosate in Drinking Water by Direct Aqueous Injection HPLC with Post Column Derivatization," in preparation, Technology Applications, Inc., 1988.

2. Glaser, J. A., D. L. Foerst, G. M. McKee, S. A. Quave, and W. L. Budde, "Trace Analyses for Wastewaters", Environ. Sci. Technol., 15, 1426, 1981.

3. Cowell, J. E., "Analytical Residue Method for N-phosphonomethyl Glycine and Aminomethyl phosphonic Acid in Environmental Water," Monsanto Company, Method Number 86-63-1, 1987.

4. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation, "American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.

5. "OSHA Safety and Health Standards, General Industry," (29CRF1910). Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

6. ASTM Annual Book of Standards, Part II, Volume 11.01, D3370-82, "Standard Practice for Sampling Water", American Society for Testing and Materials, Philadelphia, PA, 1986.

7. Cowell, J. E., "Storage Stability of Glyphosate in Environmental Water," Monsanto Company, 1988.

TABLE 1. ANALYTICAL CONDITIONS AND METHOD DETECTION LIMITS FOR GLYPHOSATE

Matrix[1]	MDL,[2] µg/L
RW	6.00
GW	8.99
TW-T	5.99

Conditions:

Column: 250 x 4 mm, Bio-Rad, Aminex A-9 (Specifications as per Subsection 6.7) thermostated at 65°C.

Mobile Phase: 0.005 M KH_2PO_4 - water:methanol (24:1) buffered at pH = 1.9 (Section 7).

Elution Mode: Isocratic

Flow Rate: 0.5 mL/min.

Injection Volume: 200 µL

PCR: Calcium Hypochlorite flow rate = 0.5 mL/min., OPA solution flow rate = 0.5 mL/min., reactor temperature = 38 C.

Detector: Excitation wavelength at 340 nm and detection emission at 455 nm.

[1] RW - reagent water, GW = ground water, TW-T = tap water spiked after dechlorination treatment.

[2] All MDL data were generated from spiked samples at 25 µg/L.

TABLE 2. RECOVERY OF GLYPHOSATE IN REPRESENTATIVE DRINKING WATER MATRICES

Fortified Concentration (µg/L)	Matrix[1]	Number of Replicates	Mean Recovery %	Relative Standard Deviation %
2500	RW	8	102	1.96
	GW	8	103	1.25
	TW-T	8	99.2	1.74
700	RW	8	101	2.65
	GW	8	98.7	2.01
	TW-T	8	96.4	1.80
250	RW	8	95.6	3.91
	GW	8	101	1.77
	TW-T	8	98.0	1.75
25	RW	8	96.0	9.07
	GW	8	96.0	12.3
	TW-T	8	108	6.57

[1] RW = Reagent water, GW = Ground water, TW-T = Tap water spiked after dechlorination treatment

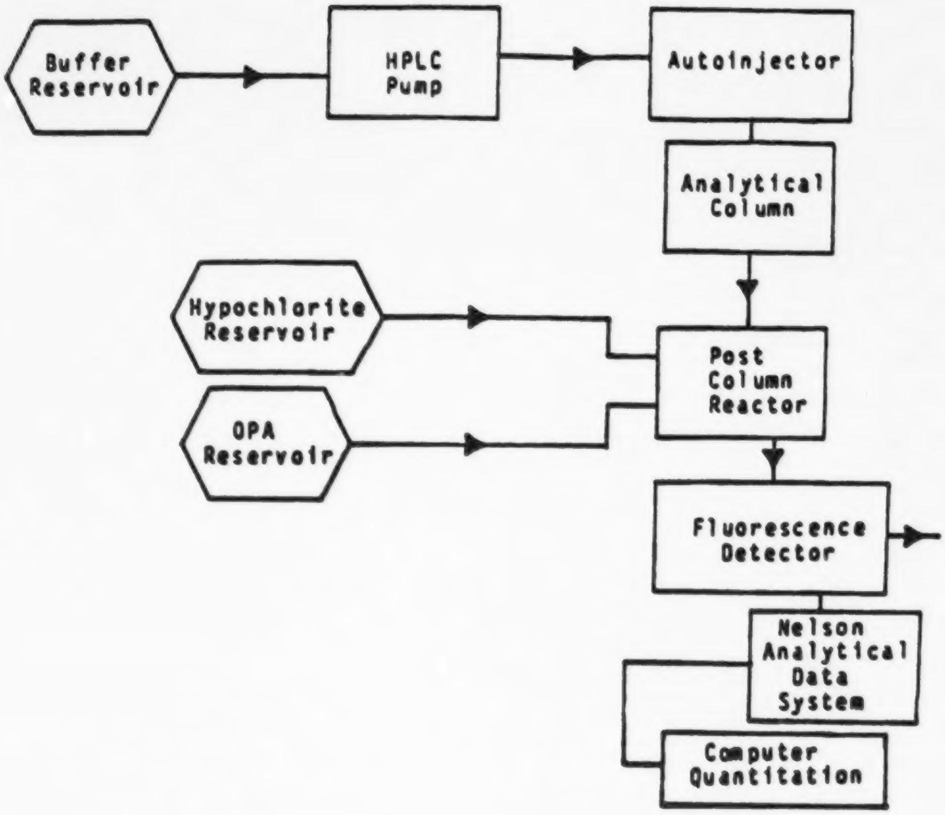
Buffer Reservoir
HPLC Pump
Autoinjector
Analytical Column
Hypochlorite Reservoir
Post Column Reactor
OPA Reservoir
Fluorescence Detector
Nelson Analytical Data System
Computer Quantitation

Figure 1. HPLC, Post-Column Reactor System

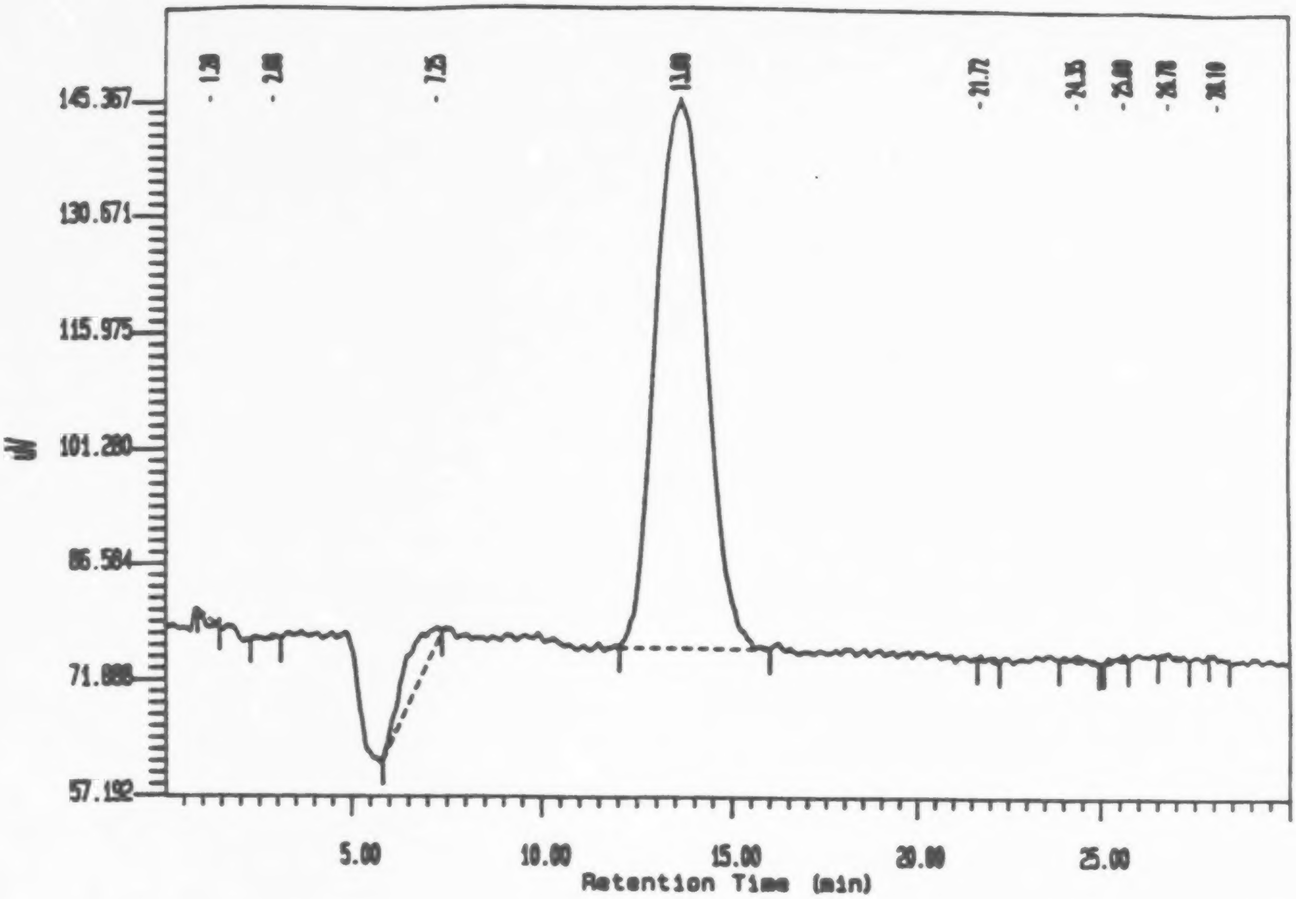

Figure 2. Liquid Chromatogram of glyphosate at 250 ug/L. Conditions are as stated in Table 1.

METHOD 548. ANALYSIS OF ENDOTHALL IN DRINKING WATER

July 1990

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METHOD 548

ANALYSIS OF ENDOTHALL IN DRINKING WATER

1. SCOPE AND APPLICATION

1.1 This method covers the determination of endothall in drinking water sources and finished drinking water. The following analyte can be determined by this method:

Analyte	Chemical Abstract Services Registry Number
Endothall	145-73-3

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above. When this method is used to analyze unfamiliar samples, compound identification should be supported by at least one additional qualitative technique. A gas chromatograph/mass spectrometer (GC/MS) may be used for the qualitative confirmation of results for Endothall using the extract produced by this method.

1.3 The method detection limit[1] (MDL, defined in Section 12) for endothall is listed in Table 1. The MDL for a specific sample may differ from the listed value, depending upon the nature of interferences in the sample matrix and the amount of sample used in the procedure.

1.4 Any modification of this method beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 11.

2. SUMMARY OF METHOD

2.1 A 5.0 mL volume of liquid sample is placed in a Kuderna-Danish tube and the volume is reduced to less than 0.75 mL using a heating block. The tube is charged with glacial acetic acid and sodium acetate, followed by a solution of the derivatization reagent, pentafluorophenylhydrazine (PFPH), in glacial acetic acid. After heating at 150°C for 90 minutes the derivative is extracted by a solid sorbent from the reaction solution, followed by elution with 5.0 mL of methyl-tert-butyl ether (MTBE). The MTBE extract is analyzed by gas chromatography with electron capture detection (GC/ECD).

3. DEFINITIONS

3.1 INTERNAL STANDARD - A pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be analyte that is not a sample component.

3.2 SURROGATE ANALYTE - A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.

3.3 LABORATORY DUPLICATES (LD1 and LD2) - Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 FIELD DUPLICATES (FD1 and FD2) - Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.5 LABORATORY REAGENT BLANK (LRB) - An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 FIELD REAGENT BLANK (FRB) - Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 LABORATORY PERFORMANCE CHECK SOLUTION (LPC) - A solution of method analytes, surrogate compounds, and internal standards used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

3.8 LABORATORY FORTIFIED BLANK (LFB) - An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.

3.9 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) - An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.10 STOCK STANDARD SOLUTION - A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.11 PRIMARY DILUTION STANDARD SOLUTION - A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.12 CALIBRATION STANDARD (CAL) - A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.13 QUALITY CONTROL SAMPLE (QCS) - A sample matrix containing method analytes or a solution of method analytes in a water miscible solvent which is used to fortify reagent water or environmental samples. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance with externally prepared test materials.

4. INTERFERENCES

4.1 Method interference may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 10.2.

4.1.1 Glassware must be scrupulously clean[2]. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. It should then be drained dry, and heated in a laboratory oven at 40°C for several hours before use. Solvent rinses with methanol may be substituted for the oven heating. After drying and cooling, glassware should be stored in a clean environment to prevent any accumulation of dust or other contaminants.

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

4.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the matrix being sampled. If significant interferences occur in subsequent samples, some additional cleanup may be necessary to achieve the MDL listed in Table 1.

4.3 The extent of interferences that may be encountered using gas chromatographic techniques has not been fully assessed. Although the GC conditions described allow for a unique resolution of the specific compound covered by this method, other matrix components may interfere.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemical specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additionally references to laboratory safety are available.

6. APPARATUS AND MATERIALS

6.1 SAMPLING EQUIPMENT (for discrete or composite sampling).

6.1.1 Grab sample bottle - Amber glass fitted with screw caps lined with Teflon. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with methanol, and dried before use to minimize contamination

6.2 GLASSWARE

6.2.1 Volumetric flasks - 5 mL, 25 mL

6.2.2 Vials - glass, 1 mL, with Teflon-lined caps

6.2.3 Glass syringes, 250 µL, 500 µL

6.2.4 Pipets - 1 mL, 4 mL

6.3 BALANCE - analytical, capable of accurately weighing 0.0001 g.

6.4 SOLID SORBENT CARTRIDGES - C18, Baker 7020-6 or equivalent

6.5 Vacuum manifold for extraction using solid sorbent cartridges - Supelco 5-7030 or equivalent

6.6 Kuderna-Danish (K-D) concentrator tubes - 10 or 25 mL graduated (Kontes K-570050-1025 or K-570050-2525)

6.6.1 Snyder column, Kuderna-Danish -2- ball micro (Kontes, K-569001-0219)

6.7 Tube heater for 25 mL K-D tubes - Kontes

6.8 Boiling chips - carborundum, #12 granules (Arthur H. Thomas Co. #1590-033 or equivalent). Heat at 400 C for 30 minutes prior to use. Cool and stored in dessicator.

6.9 Gas chromatographic system capable of temperature programming

6.9.1 Autosampler

6.9.2 Electron capture detector

6.9.3 Column: Supelco SPB-5, 0.25 mm x 30 m or equivalent

6.9.4 Strip-chart recorder compatible with detector. Use of a data system with printer for measuring and recording peak areas and retention times is recommended.

7. REAGENTS AND SOLUTIONS

7.1 REAGENT WATER - reagent water is defined as a water of very high purity, equivalent to distilled in glass solvents

7.2 PENTAFLUOROPHENYLHYDRAZINE (PFPH) - Aldrich

7.3 SODIUM ACETATE - anhydrous, Aldrich

7.4 SODIUM THIOSULFATE - Baker

7.5 ACETIC ACID - glacial, Baker

7.6 METHYL-TERT-BUTYL ETHER (MTBE) - distilled in glass (Burdick & Jackson)

7.7 ENDOTHALL-PFPH DERIVATIVE - See Appendix for synthesis procedure

7.8 ENDOSULFAN I - USEPA Repository

7.9 ENDOTHALL, monohydrate - USEPA Repository

7.10 STOCK STANDARD SOLUTIONS

7.10.1 Endothall - 10 μg/mL in reagent water

7.10.2 Endothall - 50 μg/mL in reagent water

7.10.3 Stock standard solutions must be replaced after six months, or sooner, if comparison with check standards indicates a problem.

7.11 REACTION SOLUTIONS

7.11.1 PFPH solution - 4 mg/mL in glacial acetic acid

7.11.2 Internal standard stock solution - 10 μg/mL endosulfan I in MTBE

8. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

8.1 Grab samples must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon tubing and other potential sources of contamination.

8.2 The samples must be iced or refrigerated at 4°C from the time of collection until derivatization. The analyte measured here is not known to be light sensitive, but excessive exposure to light and heat should be avoided.

8.3 Some samples are likely to be biologically active and the stability of samples upon storage will be different for each matrix. All samples should be derivatized within 7 days of collection, and analysis completed within 1 day of derivatization. If these criteria are not met, the analyst must demonstrate the stability of the stored sample by performing suitable holding time studies.

9. CALIBRATION

9.1 Establish gas chromatographic operating parameters to produce a retention time equivalent to that indicated in Table 1. The chromatographic system can be calibrated using the internal standard technique (Section 9.2)

9.1.1 Due to the complex nature of the sample chromatogram, the analyst should periodically inject a solution containing only pure endothall-PFPH (See Appendix) to verify the retention time of the derivative.

9.2 INTERNAL STANDARD CALIBRATION PROCEDURE:

9.2.1 Use 250 and 500 µL syringes to add sufficient quantities of 7.10.1 or 7.10.2 stock solutions to reagent water in 25 mL volumetric flasks to produce endothall standard solutions at the following concentrations in µg/L: 500 (250 µL of 7.10.2 stock), 200 (100 µL of 7.10.2 stock), 100 (50 µL of 7.10.2 stock) and 50 (125 µL of 7.10.1 stock).

9.2.2 Process each standard as per Section 11.2. It is recommended that triplicate samples of each standard be processed.

9.2.3 Before analyzing matrix samples, the analyst must process a series of calibration standards to validate elution patterns and the absence of interferences from reagents.

9.2.4 Analyze each calibration standard and tabulate the ratio of the peak area of the endothall-PFPH derivative versus that of the internal standard against endothall concentration. The results may be used to prepare a calibration curve for endothall.

9.2.5 The working calibration curve must be verified on each working day by processing and analyzing one or more calibration standards. If the response varies from the previous response by more than ± 20%, the test must be repeated using a fresh calibration standard. Should the retest fail, a new calibration curve must be generated.

10. QUALITY CONTROL

10.1 Each laboratory that uses this method is required to operate a formal quality control (QC) program. The minimum QC requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified blanks, laboratory fortified matrix samples and QC check standards.

10.2 LABORATORY REAGENT BLANKS. Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is analyzed or reagents are changed, a method blank must be analyzed. For this method, the method blank is filtered reagent water. If within the retention time window of an analyte of interest, the method blank produces a peak which prevents the measurement of that analyte, determine the source of contamination and eliminate the interference before processing samples.

10.3 INITIAL DEMONSTRATION OF CAPABILITY

10.3.1 Select a representative fortified concentration (about 10 times MDL) for endothall. Prepare a concentrate (in reagent water) containing the analyte at 10 times the selected concentration. Using a pipet, add 1.00 mL of the concentrate to each of at least four 10 mL aliquots of reagent water and analyze each aliquot according to procedures beginning in Section 11.

10.3.2 The recovery value should for at least three out of four consecutively analyzed samples fall in the range of R ± 30% (or within R ± $3S_R$, if broader) using the values for R and S_R for reagent water. If the recovery value meets the acceptance criteria, performance is acceptable and sample analysis may begin. If the recovery value fails these criteria, initial demonstration of capability should be repeated.

10.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples by a new, unfamiliar method prior to evidencing a basal level of skill at performing the technique. It is expected that as laboratory personnel gain experience with this method the quality of the data will improve beyond the requirements stated in Section 10.3.2.

10.4 The analyst is permitted to modify GC columns, GC conditions, or detectors to improve separations or lower analytical costs. Each time such method modifications are made, the analyst must repeat the procedures in Section 10.3.

10.5 Assessing the Internal Standard - In using the IS calibration procedure, the analyst is expected to monitor the IS response (peak area or peak height) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from the calibration standard IS response by more than 30%.

10.5.1 If a deviation of greater than 30% is encountered for a sample, re-inject the extract.

10.5.1.1 If acceptable IS response is achieved for the re-injected extract, then report the results for that sample.

10.5.1.2 If a deviation of greater than 30% is obtained for the re-injected extract, analysis of the sample should be repeated beginning with Section 11, provided the sample is still available. Otherwise, report results obtained from the re-injected extract, but annotate as suspect.

10.5.2 If consecutive samples fail the IS response acceptance criterion, immediately analyze a calibration check standard.

10.5.2.1 If the check standard provides a response factor (RF) within 20% of the predicated value, then follow procedures itemized in Section 10.5.1 for each sample failing the IS response criterion.

10.5.2.2 If the check standard provides a response factor (RF) with deviates more than 20% of the predicted value, then the analyst must recalibrate, as specified in Section 9.2.

10.6 ASSESSING LABORATORY PERFORMANCE

10.6.1 The laboratory must analyze at least one LFB per sample set (all samples analyzed within a 24 hour period). The fortifying concentration in the LFB should be 10 times the MDL. Calculate accuracy as percent recovery (X_i). If the recovery falls outside the control limits (See Section 10.6.2), the system is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.

10.6.2 Until sufficient LFB data become available, usually a minimum of results from 20 to 30 analyses, the laboratory should assess its performance against the control limits described in Section 10.3.2. When sufficient laboratory performance data becomes available, develop control limits from the mean percent recovery (X) and standard deviation (S) of the percent recovery. These data are used to establish upper and lower control limits as follows:

Upper Control Limit = X + 3S
Lower Control Limit = X - 3S

After each group of five to ten new recovery measurements, control limits should be recalculated using only the most recent 20 to 30 data points.

10.6.3 It is recommended that the laboratory periodically determine and document its detection limit capabilities for endothall.

10.6.4 Each quarter the laboratory should analyze QCS (if available). If criteria provided with the QCS are not met, corrective action should be taken and documented.

10.7 ASSESSING ANALYTE RECOVERY

10.7.1 The laboratory must add a known fortified concentration to a minimum of 10% of the routine samples or one fortified sample per set, whichever is greater. The fortified concentration should not be less than the background concentration of the sample selected for spiking. The fortified concentration should be the same as that used for the LFB (Section 10.6).

Over time, samples from all routine sample sources should be fortified.

10.7.2 Calculate the percent recovery (R_i) for each analyte, corrected for background concentrations measured in the unfortified sample, and compare these values to the control limits established in Section 10.6.2 for the analyses of LFBs.

10.7.3 If the recovery of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Section 10.6), the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample must be labelled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

11. PROCEDURE

11.1 CLEANUP AND SEPARATION - Cleanup procedures may not be necessary for a relatively clean samples matrix. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must demonstrate that the recovery of endothall is within the limits specified by the method.

11.1.1 If the sample is not clean, or the complexity is unknown, the entire sample should be centrifuged at 2500 for 10 minutes. The supernatant is decanted from the centrifuge bottle and passed through glass fiber filter paper into a container which can be tightly sealed.

11.1.2 Store all samples at 4°C.

11.2 SAMPLE EXTRACTION AND ANALYSIS

11.2.1 Measure out a 5.0 mL aliquot of the sample and place it in a 25 mL K-D tube. Add boiling chips.

11.2.2 Place on tube heater at maximum setting and concentrate sample to less than 0.5 mL.

11.2.3 Add 4 mL glacial acetic acid, 200 mg sodium acetate and 1 mL of glacial acetic acid containing 4 mg PFPH. Use glass stirring rod to break-up the sodium acetate solid. Place a Micro Snyder column on each K-D tube.

11.2.4 Heat at 150 C for 90 minutes.

11.2.5 Dilute the reaction mixture with 50 mL reagent water. Wash the residue in the tube into the aqueous solution.

11.2.6 Assemble the vacuum manifold. Rinse the solid sorbent cartridge by passing 5 mL of reagent water though the cartridge. Discard the water. Extract the aqueous sample from 12.5 by passing the sample through the solid sorbent cartridge at a rate of 5-6 mL per minute.

11.2.7 Wash the cartridge with 5 mL reagent water. Elute the cartridge with two 2 mL aliquots of MTBE. Combine the eluates with .05 mL of the internal standard stock solution (7.11.2) and dilute to 5 mL in a volumetric flask with MTBE.

11.2.8 Analyze the eluates by GC/ECD using conditions described in Table 1. This table includes the retention time and MDL that were obtained under these conditions. Sample chromatograms of an endothall standard and a LRB both with internal standard are represented in Figures 1 and 2. Other columns, chromatographic conditions, or detectors may be used if the requirements of Section 10.3 are met.

11.3 IDENTIFICATION OF ANALYTES

11.3.1 Identify a sample component by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound, then identification is considered positive.

11.3.2 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.3.3 Identification requires expert judgement when sample components are not resolved chromatographically, that is, when GC peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima, or any time doubt exists over the identification of a peak as a chromatogram, appropriate techniques such as use of an alternative detector which operates on a chemical/physical principle different from that originally used, e.g., mass spectrometry, or the use of a second chromatography column must be used.

11.4 If the peak area exceeds the linear range of the calibration curve, a smaller sample volume should be used. Alternatively, the final solution may be diluted with MTBE and reanalyzed.

11.5 If the peak area measurement is prevented by the presence of interferences, further cleanup is required.

12. CALCULATIONS

12.1 Determine the peak area ratio for endothall in the injected sample.

12.1.1 Calculate the concentration of endothall injected using the calibration curve in Section 9.2. The concentration in a liquid sample can be calculated from Equation 1:

$$\text{Equation 1} \quad \text{Concentration, } \mu\text{g/L} = \frac{(A)(VF)}{(VS)}$$

where:

A = Concentration of endothall in extract, in μg/L
VF = Final volume of MTBE, in mL
VS = Sample volume, in mL

12.2 Report results as micrograms per liter. When duplicate and fortified samples are analyzed, report all data obtained with the sample results.

12.3 For samples processed as part of a set where the laboratory fortified sample recovery falls outside of the control limits established in Section 10.3, data must be labeled as suspect.

13. METHOD PERFORMANCE

13.1 METHOD DETECTION LIMITS - The MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above the background level. The estimated MDL concentration listed in Table 1 was obtained using reagent water. Similar results were achieved using representative matrices.

13.2 This method has not been tested for linearity of recovery from fortified reagent water.

13.3 In a single laboratory using dechlorinated tap and reagent water fortified matrices, the average recoveries presented in Table 2 were obtained. The standard deviation of the percent recovery is also included in Table 2.

14. REFERENCES

1. 40 CFR Part 136, Appendix B.

2. ASTM Annual Book of Standards, Part 31, D3694-78. "Standard Practices for Preparation of Sample Containers and for Preservation of Organic Constituents", American Society for Testing and Materials, Philadelphia, PA.

TABLE 1. GAS CHROMATOGRAPHY CONDITIONS AND METHOD DETECTION LIMITS

Analyte	Ret. Time (min.)	MDL (μg/L)
Endothall	42.3	11.5

GC conditions: 0.25 mm x 30 m SPB-5 column; 2 μL injection; (Split, 40:1) hold one minute at 60 C, program to 300 C at 4 C/minute, hold at 300 C for 15 minutes.

TABLE 2. SINGLE OPERATOR ACCURACY AND PRECISION

Analyte	Matrix Type	Average Percent Recovery	Standard Deviation (percent)	Fortified Conc. (μg/L)	Number of Analyses
Endothall	Reagent Water	120	25.3	15	8
		108	15.3	150	8
	Dechlorinated Tap Water	84.0	13.8	15	8
		94.0	13.3	150	8

100 mg/L sodium thiosulfate ($Na_2S_2O_3$) added to prior to fortifying with endothall

APPENDIX

Preparation of Endothall-Pentafluorophenylhydrazine

1. Prepare solution A of endothall by dissolving 0.204 g of endothall monohydrate (1.0 mmol) in 14 mL of methylene chloride and 3.6 mL of dry tetrahydrofuran (THF).
2. Prepare solution B of dicyclohexylcarbodiimide (DCC) by dissolving 0.206 g (1.0 mmol) in 3.4 mL of dry THF.
3. Mix solutions A and B and cover with a watchglass. (Note: a white precipitate will form in 3 to 5 minutes).
4. Gently stir the mixture from Step 3 with a magnetic stirrer for 4.5 hours at ambient temperature.
5. Prepare solution C by dissolving 0.206 g of DCC and 0.198 g of pentafluorophenylhydrazine (PFPH) in 18 mL of dry THF.
6. Mix solution C with the mixture from step 4, cover with a watchglass and stir the mixture overnight (16 hours) at ambient temperature.
7. Filter the mixture and dry the filtrate under reduced pressure to yield a beige powder.
8. Recrystallize the beige powder with 20 mL of warm (40°C) methanol: H2O (8:2 v/v).
9. Filter the solution from Step 8 to remove the insoluble material.
10. Allow the filtrate from Step 9 to cool to room temperature. A precipitate will form immediately upon cooling.
11. Filter and wash the precipitate formed in Step 10 with two 1 mL portions of cold methanol: H2O (8:2). Save the filtrate.
12. Allow the filtrate from Step 11 to stand overnight covered with a watchglass at ambient temperature. A precipitate will form on standing.
13. Filter and wash the precipitate from Step 12 with two 1 mL portions of cold methanol: H2O (8:2).
14. Recrystallize the off white precipitate from Step 13 with 20 mL of warm methanol: H2O (8:2). Filter the warm solution and allow the filtrate to cool, producing a white, crystalline precipitate.
15. Filter the white precipitate from Step 14, wash with two 1 mL portions of cold methanol: H2O (8:2) and dry under vacuum.

16. Determine the melting point of the precipitate of Step 15. The melting point of the endothall-pentafluorophenylhydrazine derivative is 201.0°C. If the melting point of the precipitate is not within 1.0 C of this melting point, recrystallize again as per Steps 14 - 15.

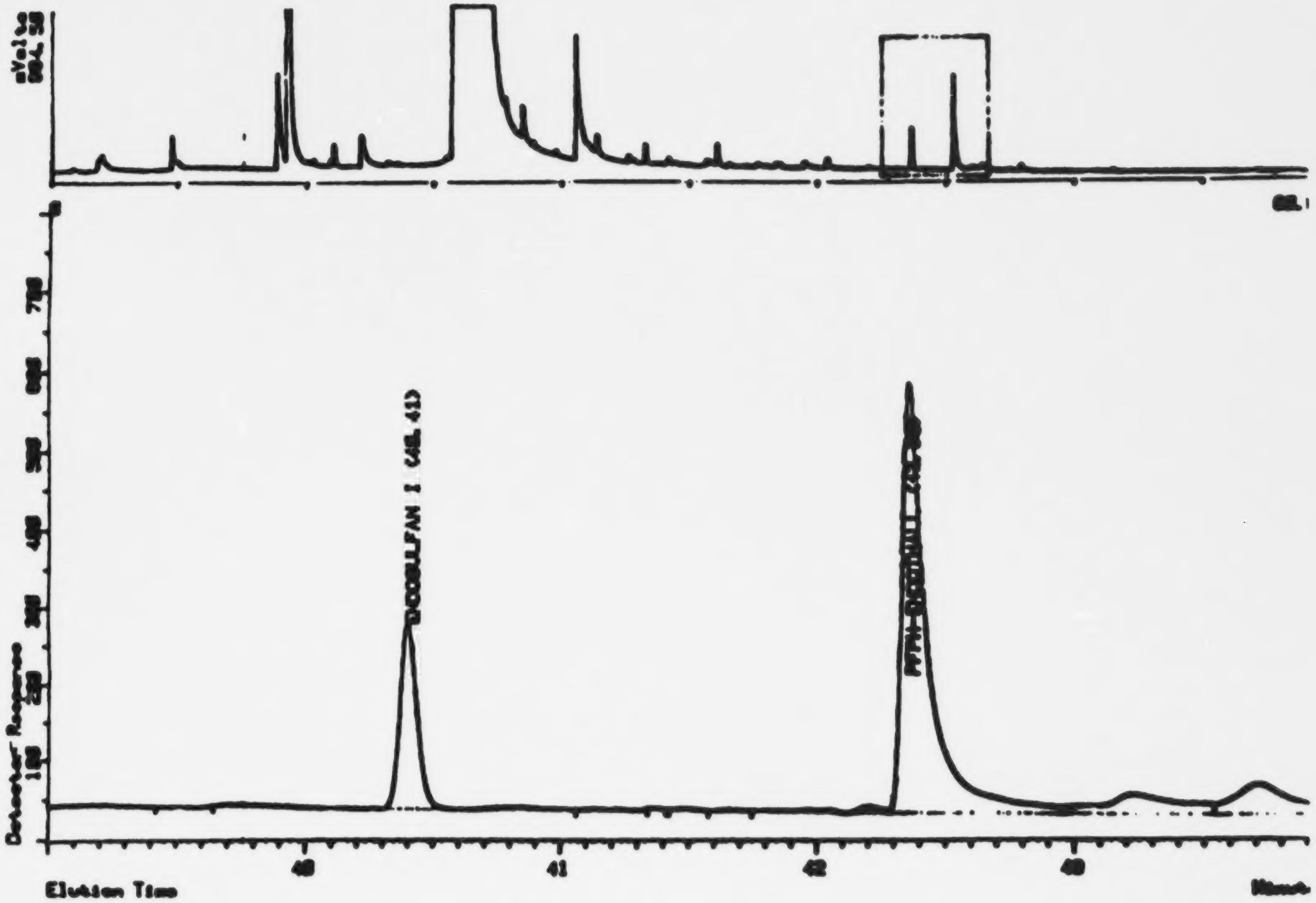

Figure 1. Representative chromatogram from injection of a 200 ug/L endothall-PFPH standard

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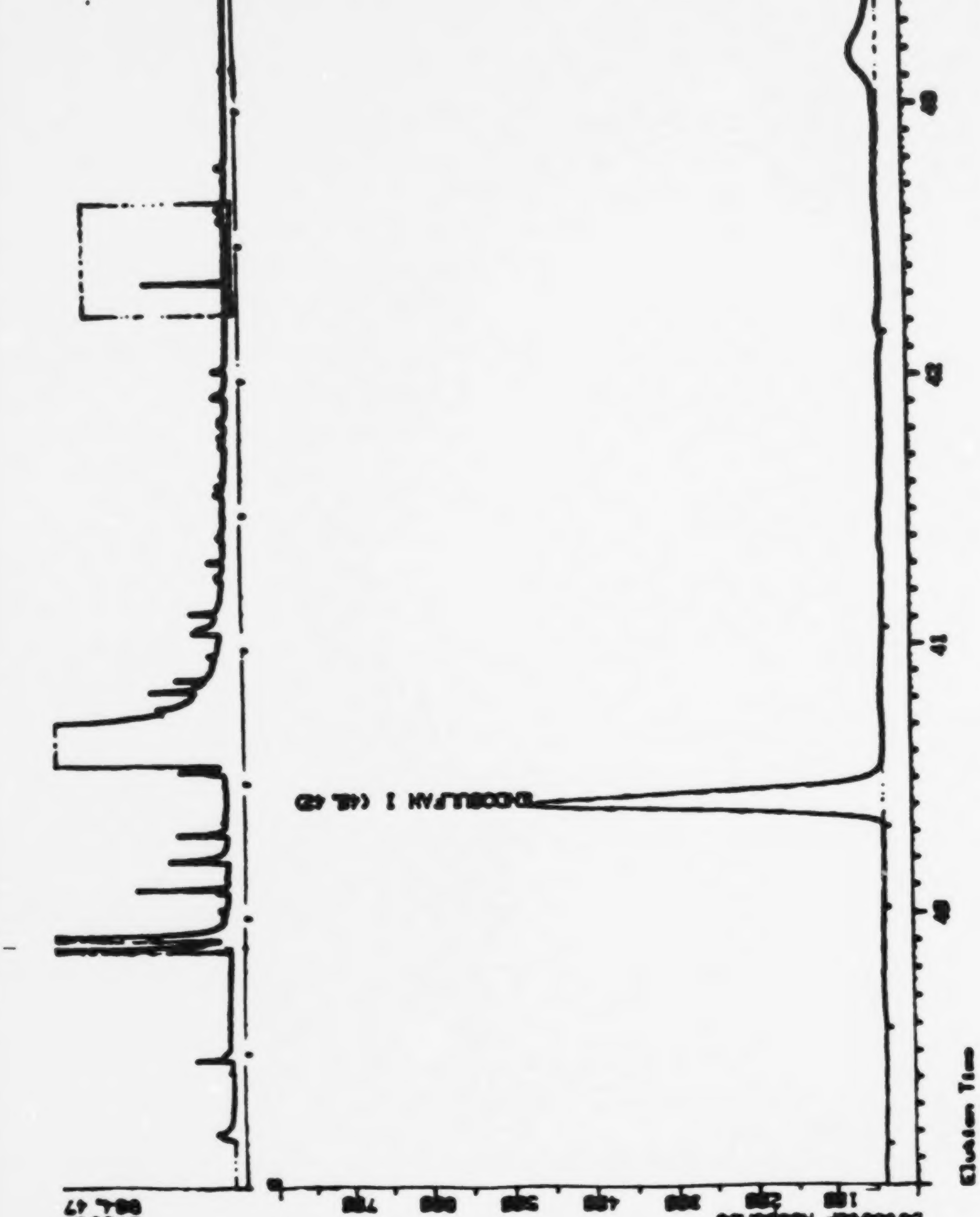

Figure 2. Representative chromatogram of a laboratory reagent blank

METHOD 549. DETERMINATION OF DIQUAT AND PARAQUAT IN DRINKING WATERS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

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METHOD 549

DETERMINATION OF DIQUAT AND PARAQUAT IN DRINKING WATERS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

1. SCOPE AND APPLICATION

1.1 This is a high performance liquid chromatography (HPLC) method for the determination of diquat (1,1'-ethylene-2,2'-bipyridilium dibromide salt) and paraquat (1,1'-dimethyl-4,4'- bipyridilium dichloride salt) in drinking water sources and finished drinking water (1,2).

Analytes	Chemistry Abstract Services Registry Number
Diquat	85-00-7
Paraquat	1910-42-5

1.2 When this method is used to analyze unfamiliar samples, compound identification should be supported by at least one additional qualitative technique. The use of a photodiode array detector provides ultraviolet spectra that can be used for the qualitative confirmation.

1.3 The method detection limits (MDL, defined in Section 13) (3) for diquat and paraquat are listed in Table 1. The MDLs for a specific sample may differ from those listed.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 10.3.

2. SUMMARY OF METHOD

2.1 A measured volume of liquid sample, approximately 250 mL, is adjusted to pH 10.5. The sample is extracted using a C_8 solid sorbent cartridge which has been specially prepared for the reversed-phase, ion-pair mode. The cartridge is eluted with 4.5 mL of an acidic aqueous solvent. After the ion-pair reagent is added to the eluate, the final volume is adjusted to 5.0 mL. Liquid chromatographic conditions are described which permit the separation and measurement of diquat and paraquat in the extract by absorbance detection at 308 nm and 257 nm, respectively. A photodiode array detector is utilized to provide simultaneous detection and confirmation of the method analytes (1,2).

2.2 Analysis of diquat and paraquat is complicated by their ionic nature. All sources of adsorption, i.e. glassware, should be avoided when possible or deactivated to prevent loss of analytes. The substitution of polyvinylchloride (PVC) for glass is recommended.

3. DEFINITIONS

3.1 LABORATORY REAGENT BLANK (LRB) - An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.2 FIELD REAGENT BLANK (FRB) - Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.3 LABORATORY FORTIFIED BLANK (LFB) - An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.

3.4 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) - An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.5 STOCK STANDARD SOLUTION - A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.6 PRIMARY DILUTION STANDARD SOLUTION -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.7 CALIBRATION STANDARD (CAL) - A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.8 QUALITY CONTROL SAMPLE (QCS) - A sample matrix containing method analytes or a solution of method analytes in a water miscible solvent which is used to fortify reagent water or environmental samples. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance with externally prepared test materials.

4. INTERFERENCES

4.1 Method interference may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatogram. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 10.2.

4.1.1 Glassware must be scrupulously cleaned (4). Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and distilled water. It should then be drained dry and heated in a laboratory oven at 130°C for several hours before use. Solvent rinses with methanol may be substituted for the oven heating. After drying and cooling, glassware should be stored in a clean environmental to prevent any accumulation of dust or other contaminants.

4.1.2 Before the initial use of all glassware, the procedure described in Section 4.1.1 should be followed. Upon drying, silanization of all glassware which will come in contact with the method analytes is necessary to prevent adsorption of the diquat and paraquat cations onto glass surfaces.

4.1.3 Plasticware should be washed with detergent and rinsed in tap water and distilled water. It should be drained dry before use.

4.1.4 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

4.2 Interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source. Because of the selectivity of the detection system used here, no interferences have been observed in the matrices studied. If interferences occur, some additional cleanup may be necessary.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be minimized. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis.

6. APPARATUS AND EQUIPMENT

6.1 SAMPLING EQUIPMENT, discrete or composite sampling.

6.1.1 Grab sample bottle - Amber polyvinylchloride (PVC) high density, one-liter, fitted with screw caps. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with deionized water, and dried before use to minimize contamination.

6.2 GlASSWARE

6.2.1 Volumetric flask - 5 mL - silanized

6.2.2 Autosampler vials - 4 mL - silanized

6.3 BALANCE - analytical, capable of accurately weighing 0.0001 g

6.4 pH METER - capable of measuring pH to 0.1 units

6.5 HPLC APPARATUS

6.5.1 Isocratic pumping system, constant flow, Waters M6000A HPLC pump or equivalent

6.5.2 Manual injector or automatic injector, capable of delivering 200 µL

6.5.3 Analytical column

6.5.3.1 Hamilton PRP-1, (5 µm, 150 mm x 4.1 mm), or equivalent

6.5.3.2 Guard column, C_8 packing

6.5.3.3 Column Oven (Fiatron, Model CH-30 and controller, Model TC-50, or equivalent)

6.5.4 Photodiode array detector (LKB 2140 Rapid Spectral Detector or equivalent) last solvent used in it. Solvent rinsing should be followed by detergent washing with hot water, and rinses with tap water and distilled water. The glassware should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Thorough rinsing with such solvents usually eliminates PCB interference. Volumetric glassware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

4.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure suggested in Section 11.1 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDLs listed in Table 1.

4.3 The extent of interferences that may be encountered using liquid chromatographic techniques has not been fully assessed. Although the HPLC conditions described allow for a unique resolution of the specific PAH covered by this method, other PAHs may interfere.

4.4 Matrix interferences have been found for benzo(a)anthracene, benzo(a)pyrene and benzo(g,h,i)perylene. Poor recovery has been experienced for these analytes in dechlorinated tap water (Table 3). The nature of the interferences has not been fully assessed.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified for the information of the analyst. (3-5)

5.2 The following analytes covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogens: benzo(a)anthracene, benzo(a)pyrene, and dibenzo(a,h)anthracene. Primary standards of these toxic compounds should be prepared in a hood. A NOISH/MESA approved toxic gas respirator should be worn when the analyst handles high concentrations of these toxic compounds.

6. APPARATUS AND EQUIPMENT (All specifications are suggested. Catalog numbers are included for illustration only.)

6.1 SAMPLING EQUIPMENT (for discrete or composite sampling).

6.1.1 Grab sample bottle - 1 L or 1 qt, amber glass, fitted with a screw cap lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The bottle and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

6.2 GLASSWARE

6.2.1 Separatory funnels - 2 L, with Teflon stopcock, 125 mL, with Teflon stopcock.

6.2.2 Drying column - Chromatographic column, approximately 250 mm long x 19 mm ID, with coarse frit filter disc.

6.2.3 Concentrator tube, Kuderna-Danish - 10 mL, graduated (Kontes K-570050-1025 or equivalent.) Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

6.2.4 Vials - 10 to 15 mL, amberglass, with Teflon-lined screw cap.

6.3 EVAPORATION EQUIPMENT

6.3.1 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

6.3.2 Nitrogen evaporation manifold - 12 port (Organomation, N-EVAP, Model III or equivalent.)

6.4 BALANCE - Analytical, capable of accurately weighing 0.0001g.

6.5 HIGH PERFORMANCE LIQUID CHROMATOGRAPH - An analytical system complete with liquid pumping system, column supplies, injector, detectors, and a compatible strip-chart recorder. A data system is highly recommended for measuring peak areas and retention times.

6.5.1 Gradient pumping system - constant flow

6.5.2 Analytical reverse-phase column - Supelco LC-PAH, 5 micron particle diameter, in a 25 cm x 4.6 mm ID stainless steel column (Supelco #5-8318 or equivalent). This column was used to develop the method performance statements in Section 13.

6.5.3 Detectors - Fluorescence and UV detectors. The fluorescence detector is used for excitation at 280 nm and emission greater than 389 nm cut-off (Schoeffel FS970 or equivalent.) Fluorometer should have dispersive optics for excitation and can utilize either filter or dispersive optics at the emission detector. The UV detector is used at 254 nm (Waters Assoc. Model 450) and should be coupled to the fluorescence detector. These detectors were used to develop the method performance statements in Section 13.

6.6 EXTRACTION APPARATUS

6.6.1 Solid Phase Extraction (SPE) cartridges, C_{18}, approximately 1000 mg/6.0 mL.

6.6.2 Solid Phase Extraction System, Baker - 10 SPE, or equivalent.

6.6.3 Vacuum pump, 100 VAC, capable of maintaining capable of maintaining a vacuum of 8-10 mm Hg.

7. REAGENTS AND CONSUMABLE MATERIALS

7.1 REAGENT WATER - Reagent water is defined as a water in which an interferant is not observed at the MDL of the analytes of interest. Prepare reagent water by filtering tap water through a bed containing ca. 0.5 kg of activated carbon, or by using commercially available water purification systems. Any source of reagent water which passes the requirements of Section 10 may be used. Store in clean bottles with teflon-lined screw caps.

7.2 SODIUM THIOSULFATE - (ACS) Granular

7.3 METHYLENE CHLORIDE - Pesticide quality or equivalent

7.4 ACETONITRILE - HPLC quality, distilled in glass

7.5 SODIUM SULFATE - (ACS) Granular, anhydrous. Purify by heating at 400°C for 4 hours in a shallow tray.

7.6 STOCK STANDARD SOLUTIONS (1.00 ug/uL) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

7.6.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. dissolve the material in acetonitrile and dilute to volume in a 10 mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is assayed at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Certified, commercially prepared stock standards can be used at any concentration.

7.6.2 Transfer the stock standard solutions into Teflon-sealed screw cap bottles. Store at 4 C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

7.6.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicates a problem.

7.7 LABORATORY CONTROL SAMPLE CONCENTRATE - See Section 10.3.1.

7.8 Fortification Solution of Internal Standards - Prepare a solution of internal standards in methanol or acetone at concentrations of 0.5 - 2.0 mg/mL. This solution may be used for the preparation of the calibration solutions specified in 9.3.1. Dilute an aliquot of the solution to 50 - 100 μg/mL and use this solution to fortify the actual water samples as directed in 11.2.2.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Grab samples must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle must not be pre-rinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon tubing and other potential sources of contamination.

8.2 All samples must be iced or refrigerated at 4°C from the time of collection until extraction. PAHs are known to be light sensitive; therefore, samples, extracts, and standards should be stored in amber or foil-wrapped bottles in order to minimize photolytic decomposition. Fill the sample bottles and, if residual chlorine is present, add 100 mg of sodium thiosulfate per liter of sample and mix well. EPA Methods 330.4 and 330.5 may be used for measurement of residual chlorine. Field test kits are available for this purpose. Adjust the pH of the sample to < 2 with 6N HCl to inhibit biological activity.

8.3 All samples must be extracted within 7 days of collection and completely analyzed within 40 days of extraction (6).

6.5.5 Data system - Use of a data system to report retention times and peak areas is recommended but not required.

6.5.5.1 IBM AT Computer with 640 KB of RAM, a 20 mb Hard Disk, and Enhanced Graphic Monitor or equivalent.

6.5.5.2 LKB Wavescan EG software, Version 1.01, to perform data reduction of spectral data or equivalent.

6.5.5.3 Printer and/or Plotter, Epson FX-286 or equivalent for report generation.

6.5.5.4 Nelson Analytical Model 2600 Software, Version 3.6 or higher, to perform integration of extracted data from Wavescan EG Software or equivalent.

6.6 EXTRACTION APPARATUS

6.6.1 Liquid Solid Extraction Cartridges, C_8, 500 mg or equivalent

6.6.2 Liquid Solid Extraction System (Baker - 10 SPE, or equivalent)

6.6.3 Vacuum pump, 100 VAC, capable of maintaining a vacuum of 8-10 mm of Hg.

6.6.4 Membrane Filters, 0.45 um pore-size, 47 mm diameter, Nylon

7. REAGENTS AND CONSUMABLES

7.1 DEIONIZED WATER - Water which has been processed through a series of commercially available filters including a particulate filter, carbon bed, ion exchange resin and finally a bacterial filter to produce deionized, reagent grade water. Any other source of reagent water may be used provided the requirements of Section 10 are met.

7.2 METHANOL - HPLC grade or higher purity

7.3 ORTHOPHOSPHORIC ACID, 85% (w/v) - reagent grade - J.T. Baker

7.4 DIETHYLAMINE - reagent grade - J.T. Baker

7.5 CONCENTRATED SULFURIC ACID - ACS reagent grade - Fisher Scientific

7.6 SODIUM HYDROXIDE - reagent grade - J.T. Baker

7.7 CONCENTRATED HYDROCHLORIC ACID, 12 N - reagent grade - J.T. Baker

7.8 CETYL TRIMETHYL AMMONIUM BROMIDE, 95% - Aldrich Chemical

7.9 SODIUM THIOSULFATE - reagent grade - J.T. Baker

7.10 1-HEXANESULFONIC ACID, sodium salt, 98%, Aldrich Chemical

7.11 1-HEPTANESULFONIC ACID, sodium salt, 98%, Aldrich Chemical

7.12 AMMONIUM HYDROXIDE, ACS, Concentrated, J.T. Baker

7.13 SYLON CT - Silanization solution - Supelco

7.14 REAGENT SOLUTIONS

7.14.1 Conditioning solution A. Dissolve 0.500 g of cetyl trimethyl ammonium bromide and 5 mL of concentrated ammonium hydroxide in 500 mL of deionized water and dilute to 1000 mL in volumetric flask.

7.14.2 Conditioning solution B. Dissolve 10.0 g of 1-hexanesulfonic acid, sodium salt and 10 mL of concentrated ammonium hydroxide in 250 mL of deionized water and dilute to 500 mL in volumetric flask.

7.14.3 Sodium hydroxide solution, 10% w/v. Dissolve 50 g of sodium hydroxide into 400 mL of deionized water and dilute to 500 mL in a volumetric flask.

7.14.4 Hydrochloric acid, 10% v/v. Add 50 mL of concentrated hydrochloric acid to 400 mL of deionized water and dilute to 500 mL in a volumetric flask.

7.14.5 Cartridge eluting solution. Add 13.5 mL of orthophosphoric acid and 10.3 mL of diethylamine to 500 mL of deionized water and dilute to 1000 mL in a volumetric flask.

7.14.6 Ion-pair concentrate. Dissolve 3.75 g of 1-hexanesulfonic acid in 15 mL of the cartridge eluting solution and dilute to 25 mL in a volumetric flask with cartridge eluting solution.

7.15 STOCK STANDARD SOLUTIONS

7.15.1 Diquat dibromide - USEPA - Pesticides and Industrial Chemical Repository, Code #2660

Paraquat dichloride - USEPA - Pesticides and Industrial Chemicals Repository, Code #5240

7.15.2 Stock diquat and paraquat solutions (1000 mg/L). Dry diquat and paraquat salts in an oven at 110°C for 3 hours. Cool in a desiccator. Repeat process to a constant weight. Weigh 0.1968 g of dried diquat salt and 0.1770 g of dried paraquat salt and place into a silanized 100 mL volumetric flask. Dissolve with approximately 50 mL of deionized water. Dilute to the mark with deionized water.

7.15.3 The salts used in preparing the stock standards (Section 7.15.2) were taken to be diquat dibromide, monohydrate and paraquat dichloride, tetrahydrate (5). The drying procedure described in Section 7.15.2 will provide these hydration levels, regardless of formulae referenced by manufacturers.

7.16 MOBILE PHASE - Make mobile phase by adding the following to 500 mL of deionized water: 13.5 mL of orthophosphoric acid; 10.3 mL of diethylamine; 3.0 g of 1-hexanesulfonic acid, sodium salt. Mix and dilute with deionized water to a final volume of 1 L.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Grab samples must be collected in either amber PVC high density bottles or silanized amber glass bottles. Conventional sampling procedures should be followed (6). Automatic sampling equipment must be free as possible of adsorption sites which might extract the sample.

8.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction. The analytes are light-sensitive, particularly diquat.

8.3 Samples which are known or suspected to contain residual chlorine must be preserved with sodium thiosulfate (100 mg/L). Samples which are biologically active must be preserved by adding sulfuric acid to a pH = 2 in order to prevent adsorption of method analytes by the humectant material.

8.4 Sample storage stability may depend on the matrix tested. Storage stability of representative drinking water matrices have been listed in Table 3. All samples must be extracted within 7 days of collection. Extracts must be analyzed within 21 days of extraction (1). If these criteria are not met, the analyst must demonstrate the stability of the stored sample by performing suitable holding times studies.

9. CALIBRATION

9.1 Establish HPLC operating conditions indicated in Table 1. The chromatographic system can be calibrated using the external standard technique.

9.2 In order to closely match calibration standards to samples, process standards by the following method: Using C_8 cartridges conditioned according to Section 11.2.1, pass 250 mL of reagent water through the cartridge and discard the water. Dry the cartridge by passing 5 mL of methanol through the cartridge. Discard the methanol. Pass 4.0 mL of the cartridge eluting solution through the cartridge and catch in a 5 mL silanized volumetric flask. Fortify the eluted solution with 100 μL of the ion-pair concentrate and with 500 μL of the stock standard and dilute to the mark with cartridge eluting solution. This provides a 10:1 dilution of the stock. Use serial dilution of the calibration standard by the same method to achieve lower concentration standards.

9.3 Analyze a minimum of three calibration standards prepared by the procedure described in Section 9.2 utilizing the HPLC conditions given in Table 1. From full spectral data obtained, extract the 308 nm chromatographic trace for diquat and the 257 nm for paraquat. Integrate and record the analyte peak areas. Any mathematical manipulations performed to aid in data reduction must be recorded and performed on all sample chromatograms. Tabulate the peak area against concentration injected. The results may be used to prepare calibration curves for diquat and paraquat.

9.4 The working calibration curve must be verified on each working day by measurement of a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (greater than 8 hr), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analyses. If the response for any analyte varies from the predicted response by more than ±20%, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve.

10. QUALITY CONTROL

10.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified matrix samples, and laboratory fortified blanks. The laboratory must maintain records to document the quality of the data generated. Additional quality control practices are recommended.

10.2 LABORATORY REAGENT BLANKS (LRB) - Before processing any samples, the analyst must analyze a LRB to demonstrate that all glassware and reagent interferences are under control. In addition, each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window (11.3.2) of the analyte of interest, the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

10.3 INITIAL DEMONSTRATION OF CAPABILITY

10.3.1 Prepare laboratory fortified blanks (LFBs) at analyte concentrations of 100 μg/L. With a syringe, add 25 μL of the stock standard (Sec. 7.14.2) to at least four - 250 mL aliquots of reagent water and analyze each aliquot according to procedures beginning in Section 11.2.

10.3.2 The glyphosate recovery (R) values determined in 10.3.1 should be within ± 30% of the R values listed in Table 2 for at least three of four consecutive samples. The relative standard deviation (S_r) of the mean recovery should be less than 30%. If the analyte of interest meets the acceptance criterion, performance is judged acceptable and sample analysis may begin. For analytes that fail this criterion, initial demonstration procedures should be repeated.

10.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. It is expected that as laboratory personnel gain experience with this method the quality of the data will improve beyond the requirements stated in Section 10.3.2.

10.4 The analyst is permitted to use other HPLC columns, HPLC conditions, or detectors to improve separations or lower analytical costs. Each time such method modifications are made, the analyst must repeat the procedures in Section 10.3.

10.5 LABORATORY FORTIFIED BLANKS

10.5.1 The laboratory must analyze at least one laboratory fortified blank (LFB) sample per sample set (all samples analyzed within a 24-h period). The fortified concentration of each analyte in the LFB should be 10 times the MDL. Calculate accuracy as percent recovery (R). If the recovery of either analyte falls outside the control limits (Section 10.5.2), that analyte is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.

10.5.2 Until sufficient data become available, usually a minimum of results from 20 to 30 analyses, the laboratory should assess laboratory performance against the control limits in Section 10.3.2. When sufficient internal performance data become available, develop control limits from the mean percent recovery (R) and standard deviation (S_r) of the percent recovery. These data are used to establish upper and lower control limits as follows:

UPPER CONTROL LIMIT = $R + 3S_r$
LOWER CONTROL LIMIT = $R - 3S_r$

After each five to ten new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points.

10.6 LABORATORY FORTIFIED SAMPLE MATRIX

10.6.1 The laboratory must add a known fortified concentration to a minimum of 10% of the routine samples or one fortified sample per set, whichever is greater. The fortified concentration should not be less then the background concentration of the original sample. Ideally, the fortified concentration should be the same as that used for the laboratory fortified blank (Section 10.5). Over time, samples from all routine samples sources should be fortified.

10.6.2 Calculate the accuracy as percent recovery (R) for each analyte, corrected for background concentrations measured in the original sample, and compare these values to the control limits established in Section 10.5.2 from the analyses of LFBs.

10.6.3 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Section 10.5), the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. The result for that analyte in the original sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

10.7 QUALITY CONTROL SAMPLES (QCS) - Each quarter the laboratory should analyze one or more QCS (if available). If criteria provided with the QCS are not met, corrective action should be taken and documented.

10.8 The laboratory may adopt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

11. PROCEDURE

11.1 SAMPLE CLEANUP - Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various sample types. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must demonstrate that the recovery of the analytes is within the limits specified by the method.

11.1.1 If the sample contains particulates, or the complexity is unknown, the entire sample should be passed through a 0.45 um

Nylon membrane filter into a silanized glass or plastic container.

11.1.2 Store all samples at 4°C unless extraction is to be performed immediately.

11.2 SAMPLE EXTRACTION AND ANALYSIS

11.2.1 Before sample extraction, the C8 extraction cartridges must be conditioned by the following procedure.

11.2.1.1 Place a C_8 cartridge on the solid phase extraction system manifold.

11.2.1.2 Elute the following solutions through the cartridge in the stated order. Take special care not to let the column go dry. The flow rate through the cartridge should be approximately 10 mL/min.

11.2.1.2.1 Cartridge Conditioning Sequence

a. Deionized water, 5 mL
b. Methanol, 5 mL
c. Deionized water, 5 mL
d. Conditioning Solution A, 5 mL
e. Deionized water, 5 mL
f. Methanol, 10 mL
g. Deionized water, 5 mL
h. Conditioning Solution B, 10 mL

11.2.1.2.2 Retain conditioning solution B in the C_8 cartridge to keep it activated.

11.2.2 The C_8 cartridges should not be prepared more than 48 hours prior to use. After conditioning, the cartridge should be capped and stored at 4°C.

11.2.3 Measure a 250 mL aliquot of the sample processed through Section 11.1 in a silanized, volumetric flask.

11.2.4 Immediately before extraction, adjust the pH of sample to 10.5 ± 0.2 with 10% w/v NaOH (aq) or 10% v/v HCl (aq).

11.2.5 Place a conditioned C_8 cartridge on the solid phase extraction vacuum manifold. Attach a 60 mL reservoir to the C_8 cartridge with the appropriate adapter. Put a 250 mL beaker inside the extraction manifold to catch waste solutions and sample. Transfer the measured volume in aliquots to the reservoir. Turn on the vacuum pump or house vacuum and adjust the flow rate to 3 to 6 mL/min. Filter the sample through the C_8 cartridge, and wash the column with

5 mL of HPLC grade methanol. Continue to draw the vacuum through the cartridge for one additional minute to dry the cartridge. Release the vacuum and discard the sample waste and methanol.

11.2.6 Place a silanized 5 mL volumetric flask beneath the collection stem in the vacuum manifold. Add 4.5 mL of the eluting solution to the sample cartridge. Turn on the vacuum and adjust the flow rate to 1 to 2 mL/min.

11.2.7 Remove the 5 mL volumetric flask with the extract. Fortify the extract with 100 µL of the ion-pair concentrate. Adjust the volume to the mark with cartridge eluting solution, mix thoroughly, and seal tightly until analyzed.

11.2.8 Analyze sample by HPLC using conditions described in Table 1. Integration and data reduction must be consistent with that performed in Section 9.3. Figure 1 presents a representative, sample chromatogram.

11.3 IDENTIFICATION OF ANALYTES

11.3.1 Identify a sample component by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds, within limits (11.3.2), to the retention time of a standard compound, then identification is considered positive.

11.3.2 The width of the retention time window used to make identification should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.3.3 Identification requires expert judgement when sample components are not resolved chromatographically. When peaks obviously represent more then one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima), or any time doubt exists over the identification of a peak in a chromatogram, a confirmatory technique must be employed. Through the use of the photodiode array detector, full spectra of the analyte peaks are obtained (Figure 2). When a peak of an unknown sample falls within the retention time windows of method analytes, confirm the peak identification by spectral comparison with analyte standards.

If additional confirmation is required, replace the 1-hexanesulfonic acid salt with 1-heptanesulfonic acid, sodium salt in the mobile phase and reanalyze the samples. Comparison of the ratio of retention times in the samples by the two mobile phases with that of the standards will provide additional confirmation.

11.3.4 If the peak area exceeds the linear range of the calibration curve, a smaller sample volume should be used. Alternatively, the final solution may be diluted with mobile phase and reanalyzed.

12. CALCULATIONS

12.1 Determine the concentration of the analytes in the sample.

12.1.1 Calculate the concentration of each analyte injected from the peak area using the calibration curves in Section 9.3 and the following equation.

$$\text{Concentration, } \mu g/L = \frac{(A) \times (VF)}{(VS)}$$

where:

A = Concentration of analyte in sample extract, in µg/L
VF = Final volume of sample extract, in mL
VS = Sample volume, in mL

12.2 Report results as micrograms per liter without correction for recovery data. When duplicate and fortified samples are analyzed, report all data obtained with sample results.

13. METHOD PERFORMANCE

13.1 METHOD DETECTION LIMITS - The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above the background level (3). The MDL data listed in Table 1 were obtained using reagent water.

13.2 This method has been tested for linearity of recovery from fortified reagent water and has been demonstrated to be applicable over the range from 4 x MDL to 1000 x MDL.

13.3 Single-laboratory precision and accuracy results at several concentration levels in drinking water matrices are presented in Table 2.

14. REFERENCES

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TABLE 1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY CONDITIONS AND METHOD DETECTION LIMITS

Analyte	Retention Time (min)	Method Detection Limits[a] (μg/L)
Diquat	2.1	0.44
Paraquat	2.3	0.80

HPLC Conditions:

Column: Hamilton PRP-1, 5μ, 4.1 mm x 150 mm

Column Temperature: 35.0 C

Flow Rate: 2.0 mL/min., Ion-Pair Mobile Phase (Section 7.16)

Injection Volume: 200 μL

LKB Photodiode Array Detector Settings:

Wavelength Range: 210 - 370 nm

Sample Rate: 1 scan/sec.

Wavelength Step: 1 nm

Integration Time: 1 sec.

Run Time: 5.0 min.

Quantitation:

Wavelengths: Diquat - 308 nm
Paraquat - 257 nm

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[a] MDL data were obtained from samples fortified at 2 μg/L (diquat) and 2.3 μg/L (paraquat), n = 6

TABLE 2. SINGLE OPERATOR ACCURACY AND PRECISION

Analyte	Matrix Type	Number of Analyses	Fortified Concentration µg/L	Relative Accuracy (Recovery) %	Relative Standard Deviation %
Diquat	Reagent Water	6	2.0	85.6	5.1
		6	10	92.1	7.3
		7	100	96.2	5.6
		7	1000	90.0	9.8
	Ground Water	6	100	102.2	3.7
	Tap[a] Water	6	100	91.3	4.7
Paraquat	Reagent Water	6	2.3	87.6	9.1
		7	11	99.7	6.9
		7	113	94.4	12.0
	Ground Water	6	113	92.1	3.4
	Tap[a] Water	6	113	74.2	1.8

[a] Dechlorinated with $Na_2S_2O_3$ (100 mg/L)

TABLE 3. 14-DAY SAMPLE HOLDING/PRESERVATION DATA[a]

Analyte	Matrix	Percent Recovery					
		Day 0		Day 7		Day 14	
		R	S_R	R	S_R	R	S_R
Diquat	RW[b]	98.8	± 8.6	93.2	± 1.4	101.9	± 2.9
	TW[c]	84.1	± 1.0	94.1	± 5.8	94.4	± 12.0
	GW[d]	84.9	± 6.6	87.5	± 3.1	72.4	± 4.5
Paraquat	RW	90.8	± 4.4	86.8	± 4.4	89.2	± 3.9
	TW	72.1	± 0.8	86.7	± 4.7	84.7	± 2.9
	GW	98.1	± 1.4	72.5	± 4.8	66.4	± 7.9

[a] Data is average of 4 samples for each matrix. All matrices were preserved with H_2SO_4 (pH = 2). Concentration of each analyte was 100 µg/L.

[b] RW = Reagent Water

[c] TW = Tap Water - Dechlorinated with $Na_2S_2O_3$ (100 mg/L)

[d] GW = Groundwater

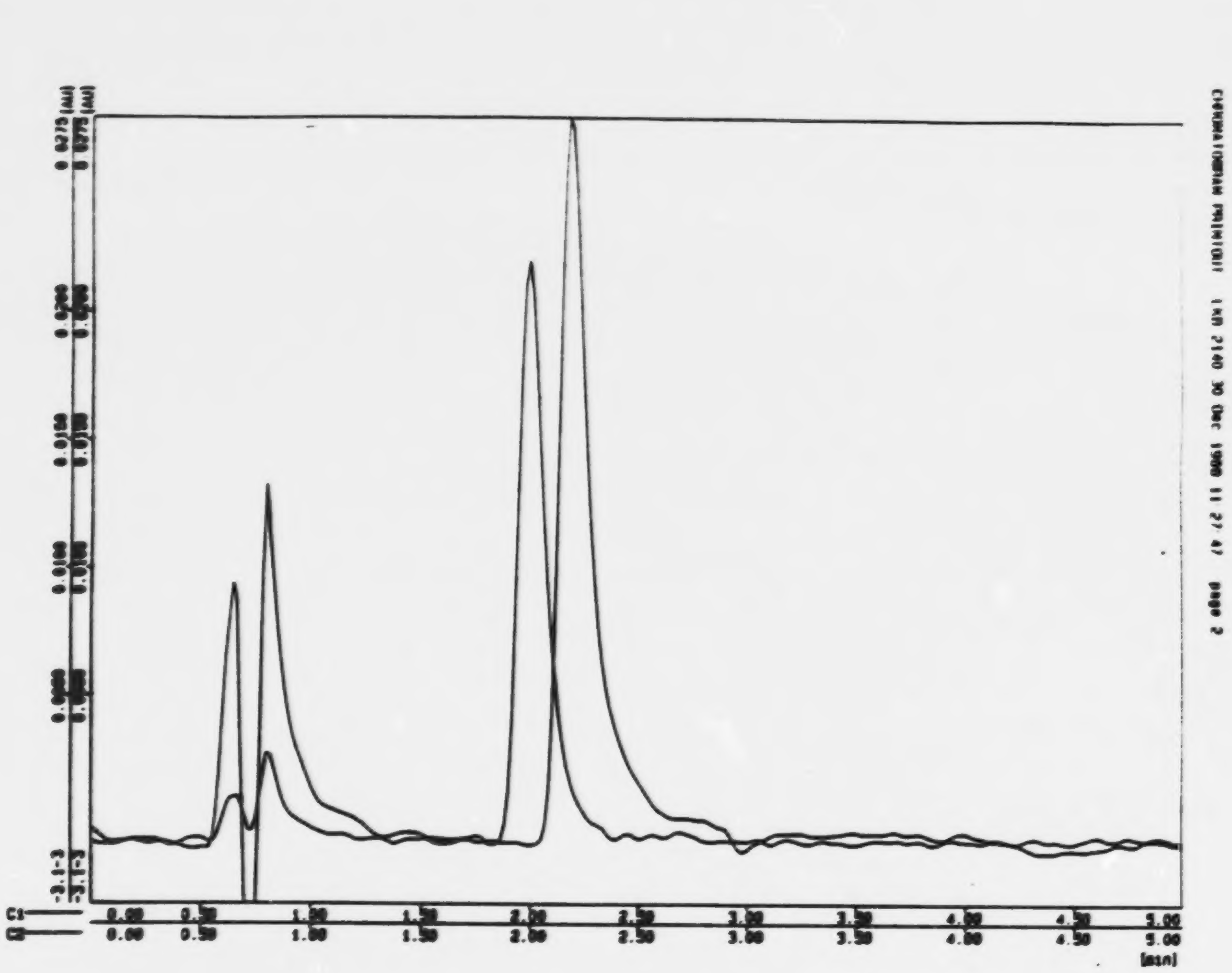

Figure 1. HPLC sample chromatograms of diquat (λ = 308 nm) and paraquat (λ = 257 nm). Retention time of diquat (C = 10 ug/L) is 2.03 min.; retention time of paraquat (C = 11 ug/L) is 2.25 min.

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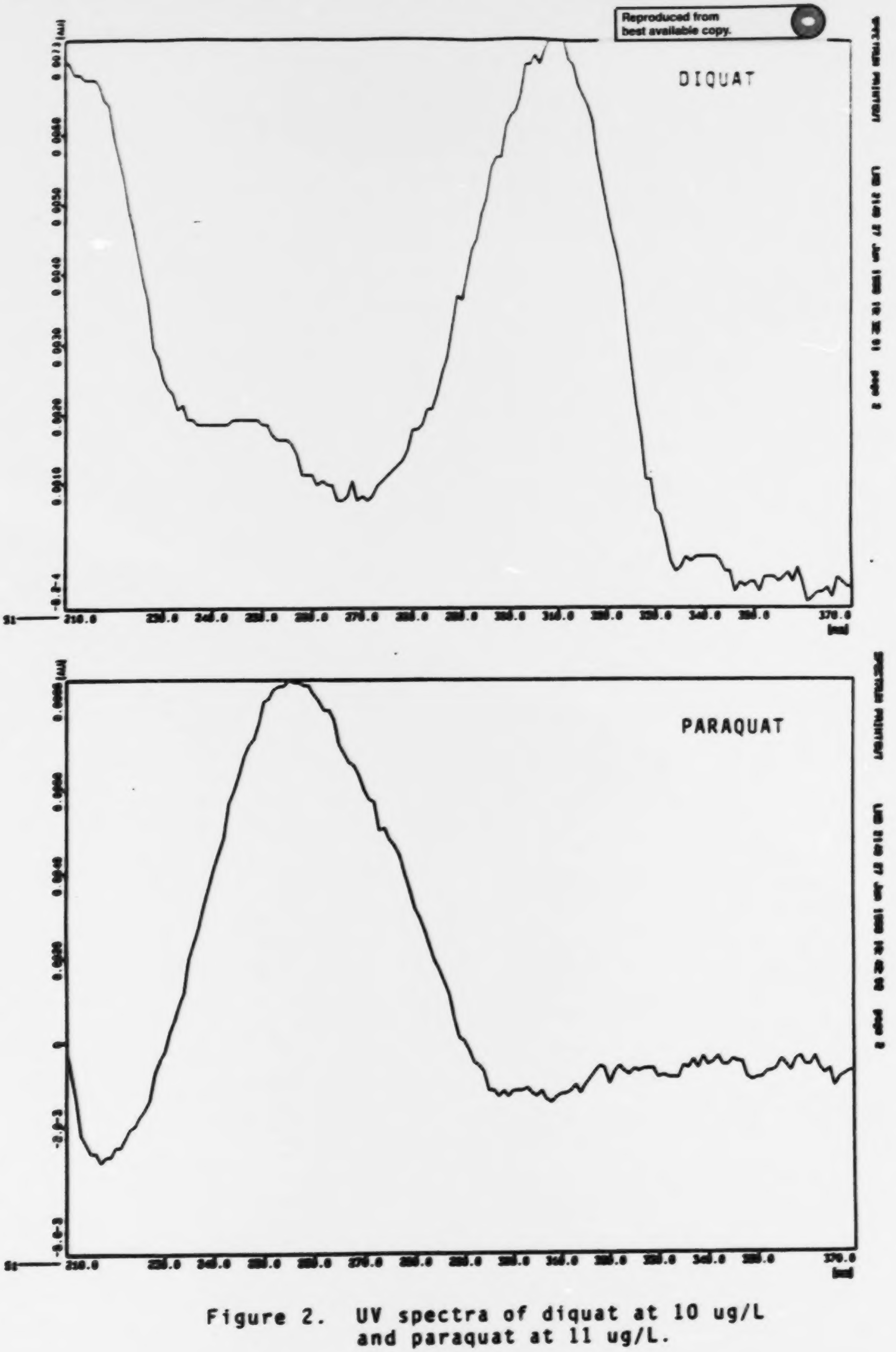

Figure 2. UV spectra of diquat at 10 ug/L
and paraquat at 11 ug/L.

METHOD 550. DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN DRINKING WATER BY HPLC LIQUID-LIQUID EXTRACTION

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METHOD 550

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN DRINKING WATER BY HPLC LIQUID-LIQUID EXTRACTION

1. SCOPE AND APPLICATION

1.1 This method describes a procedure for determination of certain polycyclic aromatic hydrocarbons (PAH) in drinking water sources and finished drinking water. The following analytes can be determined by this method:

Analyte	Chemical Abstract Services Registry Number
Acenaphthene	83-32-9
Acenaphthylene	208-96-8
Anthracene	120-12-7
Benzo(a)anthracene	56-55-3
Benzo(a)pyrene	50-32-8
Benzo(b)fluoranthene	205-99-2
Benzo(g,h,i)perylene	191-24-2
Benzo(k)fluoranthene	207-08-9
Chrysene	218-01-9
Dibenzo(a,h)anthracene	53-70-3
Fluoranthene	206-44-0
Fluorene	86-73-7
Indeno(1,2,3-cd)pyrene	193-39-05
Naphthalene	91-20-3
Phenanthrene	85-01-8
Pyrene	129-00-0

1.2 This is a high performance liquid chromatography (HPLC) method applicable to the determination of the compounds listed above. When this method is used to analyze unfamiliar samples, compound identifications should be supported by at least one qualitative technique. Method 525 provides gas chromatographic/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for the above analytes, using the extract produced by this method.

1.3 The method detection limit(1) (MDL, defined in Section 13) for each analyte is listed in Table 1. The MDL for a specific matrix may differ from those listed, depending on the nature of interferences in the sample matrix.

2. SUMMARY OF METHOD

2.1 A measured volume of sample, approximately 1 L, is serially extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to a volume of 1 mL. A

3.0 mL portion of acetonitrile is added to the extract and concentrated to a final volume of 0.5 mL. The extract analytes are then separated by HPLC. Ultraviolet adsorption (UV) and fluorescence detectors are used with HPLC to quantitatively measure the PAHs.

3. DEFINITIONS

3.1 INTERNAL STANDARD - A pure analyte(s) added to a solution in known amounts(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.2 SURROGATE ANALYTE - A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.

3.3 LABORATORY DUPLICATES (LD1 and LD2) - Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 FIELD DUPLICATES (FD1 and FD2) - Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.5 LABORATORY REAGENT BLANK (LRB) - An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 FIELD REAGENT BLANK (FRB) - Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 LABORATORY FORTIFIED BLANK (LFB) - An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the method is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.

3.8 LABORATORY FORTIFIED MATRIX SAMPLE (LFM) - An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.9 STOCK STANDARD SOLUTION - A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.10 PRIMARY DILUTION STANDARD SOLUTION - A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.11 CALIBRATION STANDARD (CAL) - A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.12 QUALITY CONTROL SAMPLE (QCS) - A sample matrix containing method analytes or a solution of method analytes in a water miscible solvent which is used to fortify reagent water or environmental samples. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance with externally prepared test materials.

4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 10.2.

4.1.1 Glassware must be scrupulously cleaned(2). Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Solvent rinsing should be followed by detergent washing with hot water, and rinses with tap water and distilled water. The glassware should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as

PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Thorough rinsing with such solvents usually eliminates PCB interference. Volumetric glassware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

4.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure suggested in Section 11.1 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDLs listed in Table 1.

4.3 The extent of interferences that may be encountered using liquid chromatographic techniques has not been fully assessed. Although the HPLC conditions described allow for a unique resolution of the specific PAH covered by this method, other PAHs may interfere.

4.4 Matrix interferences have been found for benzo(a)anthracene, benzo(a)pyrene and benzo(g,h,i)perylene. Poor recovery has been experienced for these analytes in dechlorinated tap water (Table 3). The nature of the interferences has not been fully assessed.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified for the information of the analyst.(3-5)

5.2 The following analytes covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogens: benzo(a)anthracene, benzo(a)pyrene, and dibenzo(a,h)anthracene. Primary standards of these toxic compounds should be prepared in a hood. A NOISH/MESA approved toxic gas respirator should be worn when the analyst handles high concentrations of these toxic compounds.

6. **APPARATUS AND EQUIPMENT** (All specifications are suggested. Catalog numbers are included for illustration only).

6.1 SAMPLING EQUIPMENT, for discrete or composite sampling.

6.1.1 Grab sample bottle - 1 L or 1 qt, amber glass, fitted with a screw cap lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The bottle and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

6.2 GLASSWARE

6.2.1 Separatory funnels - 2 L, with Teflon stopcock, 125 mL, with Teflon stopcock.

6.2.2 Drying column - Chromatographic column, approximately 250 mm long x 19 mm ID, with coarse frit filter disc.

6.2.3 Concentrator tube, Kuderna-Danish - 10 mL, graduated (Kontes K-570050-1025 or equivalent.) Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

6.2.4 Evaporative flask, Kuderna-Danish - 500 mL (Kontes K-57001-0500 or equivalent.) Attach to concentrator tube with springs.

6.2.5 Synder column, Kuderna-Danish - Three-ball macro (Kontes K-503000-0121 or equivalent).

6.2.6 Vials - 10 to 15 mL, amberglass, with Teflon-lined screw cap.

6.2.7 Boiling chips - carborundum, #12 granules (Arthur H. Thomas Co. #1590-033 or equivalent.) Heat at 400°C for 30 minutes prior to use. Cool and store in dessicator.

6.3 EVAPORATION EQUIPMENT

6.3.1 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2 C). The bath should be used in a hood.

6.3.2 Nitrogen evaporation manifold - 12 port (Organomation, N-EVAP, Model III or equivalent.)

6.4 BALANCE - Analytical, capable of accurately weighing 0.0001g.

6.5 HIGH PERFORMANCE LIQUID CHROMATOGRAPH - An analytical system complete with liquid pumping system, column supplies, temperature controlled column oven, injector, detectors, and a compatible strip-chart recorder. A data system is highly recommended for measuring peak areas and retention times.

6.5.1 Gradient pumping system - constant flow

6.5.2 Analytical reverse-phase column - Supelco LC-PAH, 5 micron particle diameter, in a 25 cm x 4.6 mm ID stainless steel column or equivalent. This column was used to develop the method performance statements in Section 13.

6.5.3 Detectors - Fluorescence and UV detectors. The fluorescence detector is used for excitation at 280 nm and emission greater than 389 nm cut-off (Schoeffel FS970 or equivalent.) Fluorometer should have dispersive optics for excitation and can utilize either filter or dispersive optics at the emission detector. The UV detector is used at 254 nm (Waters Assoc. Model 450) and should be coupled to the fluorescence detector. These detectors were used to develop the method performance statements in Section 13.

7. REAGENTS AND CONSUMABLE MATERIALS

7.1 REAGENT WATER - Reagent water is defined as a water in which an interferant is not observed at the MDL of the analytes of interest. Prepare reagent water by passing tap water through a filter bed containing about 0.5 kg of activated carbon, or by using a water purification system. Store in clean bottles with teflon lined screw caps.

7.2 SODIUM THIOSULFATE - (ACS) Granular

7.3 METHYLENE CHLORIDE - Pesticide quality or equivalent

7.4 ACETONITRILE - HPLC quality, distilled in glass

7.5 SODIUM SULFATE - (ACS) Granular, anhydrous. Purify by heating at 400°C for 4 hours in a shallow tray.

7.6 STOCK STANDARD SOLUTIONS (1.00 ug/uL) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

7.6.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in acetonitrile and dilute to volume in a 10 mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is assayed at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Certified, commercially prepared stock standards can be used at any concentration.

7.6.2 Transfer the stock standard solutions into Teflon-sealed screw cap bottles. Store at 4 C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

7.6.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicates a problem.

7.7 LABORATORY CONTROL SAMPLE CONCENTRATE - See Section 10.3.1.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Grab samples must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle must not be pre-rinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program.

8.2 All samples must be iced or refrigerated at 4°C from the time of collection until extraction. PAHs are known to be light sensitive; therefore, samples, extracts, and standards should be stored in amber or foil-wrapped bottles in order to minimize photolytic decomposition. Fill the sample bottles and, if residual chlorine is present, add 100 mg of sodium thiosulfate per liter of sample and mix well. EPA Methods 330.4 and 330.5 may be used for measurement of residual chlorine. Field test kits are available for this purpose. Adjust the pH of the sample to < 2 with 6N HCl to inhibit biological activity.

8.3 All samples must be extracted within 7 days of collection and completely analyzed within 40 days of extraction.(6)

9. CALIBRATION

9.1 Use liquid chromatographic operating conditions given in Table 1. The chromatographic system can be calibrated using the external standard technique (Section 9.2) or the internal standard technique (Section 9.3.) Note: Calibration standard solutions must be prepared such that no unresolved analytes are mixed together. Special care must be taken so that analyte concentrations in standard solutions are not so high as to cause peak fusing or overlap.

9.2 EXTERNAL STANDARD CALIBRATION PROCEDURE:

9.2.1 Prepare calibration standards at a minimum of three (recommend five) concentration levels for each analyte by adding volumes of one or more primary dilution standard solutions (3.10) to a volumetric flask and diluting to volume with acetonitrile. One of the external standards should be at a concentration near, but above the MDL (Table 1) and the other concentrations should bracket the expected range of concentrations found in real samples or should define the working range of the detector.

9.2.2 Using injections of 5 to 100 µL, analyze each calibration standard according to Section 11.3. Tabulate peak area or height responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected. (calibration factor) is a constant over the working range [< 10% relative standard deviation (RSD)], linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

9.3 INTERNAL STANDARD (IS) CALIBRATION PROCEDURE - To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

9.3.1 Prepare calibration standards at a minimum of three (recommend five) concentration levels for each analyte of interest by adding volumes of one or more primary dilution standard solutions (3.10) to a volumetric flask. To each calibration standard, add a known amount of one or more internal standards, and dilute to volume with acetonitrile. One of the standards should be at a concentration near but above, the MDL and the other concentrations should bracket the analyte concentrations found in the sample concentrates or should define the working range of the detector.

9.3.2 Using injections of 5 to 100 µL analyze each calibration standard according to Section 11.3. Tabulate peak height or area responses against concentration for each compound and internal standard. Calculate response factor (RF) for each compound using Equation 1.

Equation 1

$$RF = \frac{[As]\,[Cis]}{[Ais]\,[Cs]}$$

where:

As = Response for the analyte to be measured
Ais = Response for the internal standard

Cis = Concentration of the internal standard (µg/L)
Cs = Concentration of the analyte to be measured (µg/L)

If RF value over the working range is constant (< 10% RSD), the RF can be assured to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, As/Ais vs. Cs/Cis.

9.4 The working calibration curve, calibration factor, or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any analyte varies from the predicted response by more than ± 20%, the test must be repeated using fresh calibration standard. If the fresh calibration standard also deviates by more ± 20%, a new calibration curve must be prepared for that compound.

9.4.1 Daily calibration requirements using the external standard calibration procedure are a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (> 8 hrs), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analysis.

9.4.2 Minimum daily calibration requirements using the internal standard calibration procedure consist of initial analyses of a calibration check standard followed by verification of the internal standard response of each sample applying criterion described in Section 10.4.

9.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from reagents.

10. QUALITY CONTROL

10.1 Each laboratory that uses this method is required to operate a formal quality control (QC) program. The minimum QC requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified blanks, laboratory fortified matrix samples and QC samples. The laboratory must maintain records to document the quality of the data generated. Additional quality control practices are recommended.

10.2 LABORATORY REAGENT BLANKS (LRB). Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is analyzed or reagents are changed, a LRB must be analyzed. For this method, the LRB is filtered reagent water. If within the retention time window (11.4.2) of an analyte of interest, the LRB produces a peak that interferes with analyte determination, determine the source of contamination and eliminate the interference before processing samples.

10.3 INITIAL DEMONSTRATION OF CAPABILITY

10.3.1 Select a representative spike concentration (about 10 times MDL) for each analyte. Prepare a laboratory control sample concentrate (in acetonitrile) from the stock standard solution containing each analyte at 1000 times the selected concentration. Using a pipet, add 1.00 mL of the concentrate to each of at least four 1 L aliquots of reagent water and analyze each aliquot according to procedures beginning in Section 11.2.

10.3.2 For each analyte, the recovery value must for at least three out of four consecutively analyzed samples fall in the range of R ± 30% (or within R ± 3 S_r, if broader) using the values for R and S_r for reagent water in Table 2 (fortification level 1). The relative standard deviation of the mean recovery measured in 10.3.1 should be ≤ 30% or $3S_r$ (whichever is greater), using the values for S_r (level 1) in Table 2. For those compounds that meet the acceptance criteria, performance is acceptable and sample analysis may begin. For those compounds that fail these criteria, initial demonstration of capability must be repeated.

10.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples by a new, unfamiliar method prior to evidencing a basic level of skill at performing the technique. It is expected that as laboratory personnel gain experience with this method the quality of the data will improve beyond the requirements stated in Section 10.3.2.

10.3.4 The analyst is permitted to modify HPLC columns, HPLC conditions, or detectors to improve separations or lower analytical costs. Each time such method modifications are made, the analyst must repeat the procedures in Section 10.3.

10.4 ASSESSING THE INTERNAL STANDARD - When using the IS calibration procedure, the analyst is expected to monitor the IS response (peak area or peak height) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from the daily mean IS response by more than 30%.

10.4.1 If a deviation of > 30% is encountered for a sample, re-inject the extract.

10.4.1.1 If acceptable IS response is achieved for the re-injected extract, then report the results for that sample.

10.4.1.2 If a deviation of > 30% is obtained for the re-injected extract, analysis of the sample should be repeated beginning with Section 11.2, provided the sample is still available. Otherwise, report results obtained from the re-injected extract, but annotate as suspect.

10.4.2 If consecutive samples fail the IS response acceptance criterion, immediately analyze a calibration check standard.

10.4.2.1 If the check standard provides a response factor (RF) within 20% of the predicted value, then follow procedures itemized in Section 10.4.1 for each sample failing the IS response criterion.

10.4.2.2. If the check standard provides a response factor (RF) which deviates more than 20% of the predicted value, then the analyst must recalibrate, as specified in Section 9.3.

10.5 LABORATORY FORTIFIED BLANK

10.5.1 The laboratory must analyze at least one laboratory fortified blank (LFB) per sample set (all samples analyzed within a 24 hour period). The fortified concentration of each analyte in the LFB should be at least 10 times the MDL. Calculate accuracy as percent recovery (R). If the recovery of any analyte falls outside the control limits (See Section 10.5.2), that analyte is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.

10.5.2 Until sufficient LFB data become available, usually a minimum of results from 20 to 30 analyses, the laboratory must assess its performance against the control limits described in Section 10.3.2. When sufficient laboratory performance data becomes available, develop control limits from the mean percent recovery (R) and standard deviation (S) of the percent recovery. These data are used to establish upper and lower control limits as follows:

Upper Control Limit = R + 3S
Lower Control Limit = R - 3S

After each group of five to ten new recovery measurements, control limits should be recalculated using only the most recent 20 to 30 data points.

10.6 LABORATORY FORTIFIED MATRIX SAMPLE

10.6.1 The laboratory must add a known fortified concentration to a minimum of 10% of the routine samples or one fortified sample per set, whichever is greater. The fortified concentration should not be less than the background concentration of the original sample. Ideally, the fortified concentration should be the same as that used for the LFB (Section 10.5). Over time, samples from all routine sample sources should be fortified.

10.6.2 Calculate the percent recovery (R) for each analyte, corrected for background concentrations measured in the original sample, and compare these values to the control limits established in Section 10.5.2 from the analyses of LFBs.

10.6.3 If the recovery of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Section 10.5), the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. The result for that analyte in the original sample must be labelled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

10.7 Quality Control Samples (QCS) - Each quarter the laboratory should analyze one or more QCS (if available). If criteria provided with the QCS are not met, corrective action should be taken and documented.

10.8 The laboratory may adopt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

11. PROCEDURE

11.1 **SAMPLE CLEANUP** - Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a cleanup procedure, the analyst first must demonstrate that the requirements of Section 10.5 can be met using the method as revised to incorporate the cleanup procedure. EPA Method 610 describes one possible cleanup procedure for this analyte list.

11.2 **SAMPLE EXTRACTION - LIQUID-LIQUID EXTRACTION**

11.2.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2 L separatory funnel.

11.2.2 Add 60 mL of methylene chloride to the sample bottle, seal, and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layers, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250 mL Erlenmeyer flask.

11.2.3 Add a second 60 mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

11.2.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10 mL concentrator tube to a 500 mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D concentrator if the requirements of Section 10.6 are met.

11.2.5 Pour the combined extract through a solvent-rinsed drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 - 30 mL of methylene chloride to complete the quantitative transfer.

11.2.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparatus volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

11.2.7 Remove the Synder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5 mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated (4 C) if further processing will not be performed immediately. If the extract will be stored longer than two days, it should be transferred to a Teflon-sealed screw-cap vial and protected from light.

11.2.8 Evaporate the extract with a gentle stream of N_2 flow to a volume of 1.0 mL. Add 3.0 mL of acetonitrile (MeCN) and concentrate with the N_2 flow to a final volume of 0.5 mL. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract will be stored longer than two days, it should be transferred to a Teflon-sealed screw cap vial and protected from light. If the sample extract requires no further cleanup, proceed with liquid chromatographic analysis (Section 11.3).

11.2.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11.3 SAMPLE ANALYSIS

11.3.1 Table 1 summarizes the recommended operating conditions for the HPLC. Included in this table are retention times and MDLs that can be achieved under these conditions. The UV detector is recommended for the determination of naphthalene, acenaphthylene, acenaphthene and fluorene. The fluorescence detector is recommended for the remaining PAHs. An example for the separation achieved by this HPLC column is shown in Figure 1. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 10.5 are met.

11.3.2 Calibrate the system daily as described in Section 9.

11.3.3 If the internal standard calibration procedure is being used, the internal standard must be added to the sample extract and mixed thoroughly immediately before injection into the instrument.

11.3.4 Inject 5 to 100 µL of the sample extract or standard into the HPLC using a high pressure syringe or a constant volume sample injection loop. Record the volume injected to the nearest 0.1 µL, and the resulting peak size in area or peak height units. Re-equilibrate the HPLC column at the initial gradient conditions for at least 10 minutes between injections.

11.3.5 If the response for a peak exceeds the working range of the system, dilute the extract with acetonitrile and reanalyze.

11.4 IDENTIFICATION OF ANALYTES

11.4.1 Identify a sample component by comparison of its retention time to the retention time in reference chromatogram. If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound, then identification is considered positive.

11.4.2 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.4.3 Identification requires expert judgement when sample components are not resolved chromatographically, that is, when GC peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima). Any time doubt exists over the identification of a peak in a chromatogram, appropriate confirmatory techniques need to be employed such as use of an alternative detector which operates on a chemical/physical principle different from that originally used, e.g., mass spectrometry, or the use of a second chromatography column.

12. CALCULATIONS

12.1 Determine the concentration of individual compounds in the sample as follows.

12.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor determined in Section 9.2.2. The concentration in the sample can be calculated from Equation 2.

Equation 2

$$\text{Concentration } (\mu g/L) = \frac{(A)\ (V_t)}{(V_i)\ (V_s)}$$

where:

A = Amount of material injected (ng).
Vi = Volume of extract injected (μL).

Vt = Volume of total extract (µL).
Vs = Volume of water extraction (mL).

12.1.2 If the internal standard calibration procedure is used, calculate the concentration in the sample using the response factor (RF) determined in Section 9.3.2 and Equation 3.

Equation 3

$$\text{Concentration } (\mu g/L) = \frac{(A_s)\ (I_s)}{(A_{is})\ (RF)\ (V_o)}$$

where:

As = Response for the parameter to be measured.
Ais = Response for the internal standard.
Is = Amount of internal standard added to each extract (µg).
Vo = Volume of water extracted (L).

12.2 Report results in µg/L without correction for recovery data. All QC data obtained should be reported with the sample results.

13. METHOD PERFORMANCE

13.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL is equal to the level calculated by multiplying the standard deviation of N replicate measurements times the students' t test critical value for a 99 percent confidence level at N - 1 degrees of freedom.

13.2 In a single laboratory, analyte recoveries from reagent water were determined at two concentration levels. Results were used to determine analyte MDLs and demonstrate method range. Analytes were divided into three spiking sets: compounds measured by UV detection (UV) and two groups of compounds measured by fluorescence detection (FD-A and FD-B), and analyzed separately. MDL values are given in Table 1. Precision and accuracy data obtained for the two concentration levels in reagent water are presented in Table 2.

13.3 In a single laboratory, analyte recoveries from dechlorinated tap water were determined at one concentration level. Results were used to demonstrate method performance capabilities for a finished drinking water matrix. As with Section 13.2, analytes were grouped into three spiking sets (UV, FD-A and FD-B). Precision and accuracy results for the dechlorinated tap water are shown in Table 3.

14. REFERENCES

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TABLE 1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY CONDITIONS AND METHOD DETECTION LIMITS

Analyte	Sample Set	Retention Time min	Method Detection Limit µg/L (a)	Method Detection Limit Fortification Level µg/L
Naphthalene	UV	12.5	3.3	10.0
Acenaphthylene	UV	13.8	2.3	10.0
Acenaphthene	UV	15.4	3.0	10.0
Fluorene	UV	15.6	0.25	1.00
Phenanthrene	FD-B	16.8	0.162	0.500
Anthracene	FD-A	17.6	0.079	0.625
Fluoranthene	FD-B	18.7	0.026	0.025
Pyrene	FD-A	19.4	0.126	0.625
Benzo(a)anthracene	FD-B	21.9	0.002	0.010
Chrysene	FD-A	22.3	0.063	0.625
Benzo(b)fluoranthene	FD-B	24.2	0.003	0.010
Benzo(k)fluoranthene	FD-A	25.0	0.002	0.0125
Benzo(a)pyrene	FD-B	26.0	0.029	0.050
Dibenzo(a,h)anthracene	FD-B	27.1	0.019	0.125
Benzo(g,h,i)perylene	FD-B	27.8	0.014	0.050
Indeno(1,2,3-cd)pyrene	FD-A	28.3	0.011	0.125

HPLC column conditions: Reverse-phase LC-PAH, 5 micron particle size, in a 25 cm x 4.6 mm ID stainless steel column. Isocratic elution for 2 min. using acetonitrile/water (3.5 : 6.5), then linear gradient elution to 100% acetonitrile over 22 min. at 2.0 mL/min. flow rate.

(a) The MDL for naphthalene, acenaphthylene, acenaphthene, and fluorene were determined using a UV detector. All others were determined using a fluorescence detector.

TABLE 2. SINGLE-LABORATORY ACCURACY AND PRECISION FROM SEVEN REPLICATE ANALYSES OF FORTIFIED REAGENT WATER

Analyte	Concentration Level 1 µg/L	R(a)	S_r(b)	Concentration Level 2(c) µg/L	R	S_r
Naphthalene	10.0	96.0	10.5	2.0	83.3	17.4
Acenaphthylene	10.0	95.5	7.0	2.0	98.5	15.2
Acenaphthene	10.0	94.5	9.5	2.0	47.3	6.2
Fluorene	1.0	91.0	8.0	0.2	92.0	7.8
Phenanthrene	0.5	72.5	10.3	0.1	69.5	17.0
Anthracene	0.625	89.6	4.0	0.125	74.4	13.2
Fluoranthene	0.025	113	33.2	0.005	140	50.0
Pyrene	0.625	93.6	6.4	0.125	82.4	9.6
Benzo(a)anthracene	0.01	99.0	10.5	0.002	50.0	10.0
Chrysene	0.625	94.4	3.2	0.125	94.0	11.6
Benzo(b)fluoranthene	0.01	99.0	10.5	0.002	50.0	25.0
Benzo(k)fluoranthene	0.0125	77.6	6.0	0.0025	100	40.0
Benzo(a)pyrene	0.05	85.7	18.3	0.01	41.7	16.7
Dibenzo(a,h)anthracene	0.125	81.6	4.8	0.025	78.0	12.0
Benzo(g,h,i)perylene	0.05	108	9.0	0.01	50.0	10.0
Indeno(1,2,3-cd)pyrene	0.125	72.4	2.8	0.025	66.0	8.0

(a) R = Mean Recovery, %

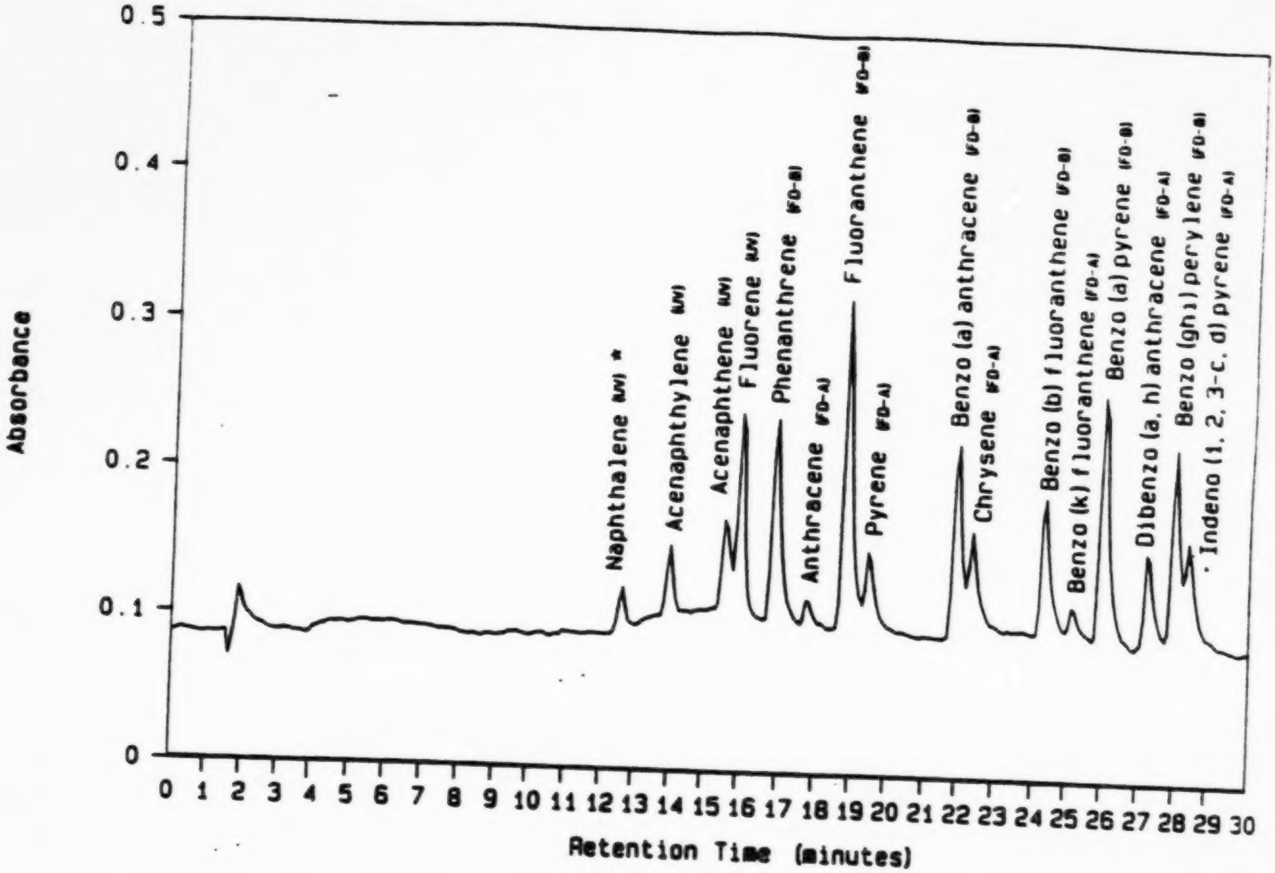
(b) S_r = Relative Standard Deviation of R, %

(c) Spike Level 2 = Concentration for analytes which yield a signal-to-noise ratio of approximately 10 in the extract (25 µL injection)

TABLE 3. SINGLE-LABORATORY ACCURACY AND PRECISION FROM SEVEN REPLICATE ANALYSES OF FORTIFIED TAP WATER (a)

Analyte	Fortified Concentration Level µg/L	Relative Accuracy (Recovery) %	Relative Standard Deviation %
Naphthalene	10.0	77.0	5.0
Acenaphthylene	10.0	82.0	4.5
Acenaphthene	10.0	90.0	7.5
Fluorene	1.0	78.0	5.0
Phenanthrene	0.5	74.5	4.9
Anthracene	0.625	93.6	6.7
Fluoranthene	0.025	98.0	10.0
Pyrene	0.625	87.2	5.4
Benzo(a)anthracene	0.01	45.0	10.0
Chrysene	0.625	92.0	6.2
Benzo(b)fluoranthene	0.01	85.0	5.0
Benzo(k)fluoranthene	0.0125	64.0	4.0
Benzo(a)pyrene	0.05	8.3	10.0
Dibenzo(a,h)anthracene	0.125	87.3	7.2
Benzo(g,h,i)perylene	0.05	54.0	12.0
Indeno(1,2,3-cd)pyrene	0.125	76.8	7.6

(a) Tap water was dechlorinated with sodium thiosulfate (100 mg/L) prior to spiking with analytes.



*(UV), (FD-A)and (FD-B) indicate analysis sets. See Section 15

Figure 1. PAH, HPLC chromatogram using UV detection. Chromatographic conditions are as stated in Table 1.

METHOD 550.1. DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN DRINKING WATER BY HPLC LIQUID-SOLID EXTRACTION

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METHOD 550.1

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN DRINKING WATER BY HPLC LIQUID-SOLID EXTRACTION

1. SCOPE AND APPLICATION

1.1 This method describes a procedure for determination of certain polycyclic aromatic hydrocarbons (PAH) in drinking water sources and finished drinking water. The following analytes can be determined by this method:

Analyte	Chemical Abstract Services Registry Number
Acenaphthene	83-32-9
Acenaphthylene	208-96-8
Anthracene	120-12-7
Benzo(a)anthracene	56-55-3
Benzo(a)pyrene	50-32-8
Benzo(b)fluoranthene	205-99-2
Benzo(g,h,i)perylene	191-24-2
Benzo(k)fluoranthene	207-08-9
Chrysene	218-01-9
Dibenzo(a,h)anthracene	53-70-3
Fluoranthene	206-44-0
Fluorene	86-73-7
Indeno(1,2,3-cd)pyrene	193-39-05
Naphthalene	91-20-3
Phenanthrene	85-01-8
Pyrene	129-00-0

1.2 This is a high performance liquid chromatography (HPLC) method applicable to the determination of the compounds listed above. When this method is used to analyze unfamiliar samples, compound identifications should be supported by at least one qualitative technique. Method 525 provides gas chromatographic/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for the above analytes, using the extract produced by this method. Note: To utilize Method 525, the standards must be in acetonitrile also.

1.3 The method detection limit(1) (MDL, defined in Section 13) for each analyte is listed in Table 1. The MDL for a specific matrix may differ from those listed, depending on the nature of interferences in the sample matrix.

2. SUMMARY OF METHOD

2.1 Polycyclic aromatic hydrocarbons and internal standards, if used, are extracted from a water sample by passing 1 liter of sample through a cartridge containing about 1 gram of a solid inorganic matrix coated with a chemically bonded C18 organic phase (liquid-solid extraction, LSE). The use of disks impregnated with the same material is also acceptable. The compounds are eluted from the cartridge or disk with a small quantity of methylene chloride, dried. and concentrated further to 1 mL. A 3.0 mL portion of acetonitrile is added to the extract and concentrated to a final volume of 0.5 mL. The extract is then separated by HPLC. Ultraviolet (UV) adsorption and fluorescence detectors are used with HPLC to identify and measure the PAHs.

3. DEFINITIONS

3.1 INTERNAL STANDARD - A pure analyte(s) added to a solution in known amounts(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.2 SURROGATE ANALYTE - A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.

3.3 LABORATORY DUPLICATES (LD1 and LD2) - Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 FIELD DUPLICATES (FD1 and FD2) - Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.5 LABORATORY REAGENT BLANK (LRB) - An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 FIELD REAGENT BLANK (FRB) - Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 LABORATORY FORTIFIED BLANK (LFB) - An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the method is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.

3.8 LABORATORY FORTIFIED MATRIX SAMPLE (LFM) - An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.9 STOCK STANDARD SOLUTION - A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.10 PRIMARY DILUTION STANDARD SOLUTION - A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.11 CALIBRATION STANDARD (CAL) - A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.12 QUALITY CONTROL SAMPLE (QCS) - A sample matrix containing method analytes or a solution of method analytes in a water miscible solvent which is used to fortify reagent water or environmental samples. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance with externally prepared test materials.

4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 10.2.

4.1.1 Glassware must be scrupulously cleaned(2). Clean all glassware as soon as possible after use by rinsing with the

9. CALIBRATION

9.1 Use liquid chromatographic operating conditions equivalent to those given in Table 1. The chromatographic system can be calibrated using the external standard technique (Section 9.2) or the internal standard technique (Section 9.3.)

9.2 EXTERNAL STANDARD CALIBRATION PROCEDURE:

9.2.1 Prepare calibration standards at a minimum of three concentration levels for each analyte by adding volumes of one or more primary dilution standard solutions (3.10) to a volumetric flask and diluting to volume with acetonitrile. One of the external standards should be at a concentration near, but above the MDL (Table 1) and the other concentrations should bracket the expected range of concentrations found in real samples or should define the working range of the detector.

9.2.2 Using injections of 5 to 100 µL, analyze each calibration standard according to Section 11.10. Tabulate peak area or height responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected, (calibration factor) is a constant over the working range [< 10% relative standard deviation (RSD)], linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

9.3 INTERNAL STANDARD (IS) CALIBRATION PROCEDURE - To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, specific internal standard are not recommended in this method. The compound, 4,4'-difluorobiphenyl is a candidate internal standard for the early eluting compounds determined by UV adsorbance. Other substituted polynuclear aromatics, or polynuclears not on the analyte list, are candidate internal standards for the higher molecular weight, fluorescent analytes.

9.3.1 Prepare calibration standards at a minimum of three concentration levels for each analyte of interest by adding volumes of one or more primary dilution standard solutions (3.10) to a volumetric flask. To each calibration standard, add a known amount of one or more internal standards, and dilute to volume with acetonitrile. One of the standards should be at a concentration near but above, the MDL and the other concentrations should bracket the analyte concentrations found in the sample concentrates or should define the working range of the detector.

9.3.2 Using injections of 5 to 100 µL analyze each calibration standard according to Section 11.3. Tabulate peak height or area responses against concentration for each compound and internal standard. Calculate response factor (RF) for each compound using Equation 1.

Equation 1

$$RF = \frac{[As]\,[Cis]}{[Ais]\,[Cs]}$$

where:

As = Response for the analyte to be measured
Ais = Response for the internal standard
Cis = Concentration of the internal standard (µg/L)
Cs = Concentration of the analyte to be measured (µg/L)

If RF value over the working range is constant (< 10% RSD), the RF can be assured to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, AS/Ais vs. Cs/Cis.

9.4 The working calibration curve, calibration factor, or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any analyte varies from the predicted response by more than ± 20%, the test must be repeated using fresh calibration standard. If the fresh calibration standard also deviates by more ± 20%, a new calibration curve must be prepared for that compound.

9.4.1 Daily calibration requirements using the external standard calibration procedure are a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (> 8 hrs), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analysis.

9.4.2 Minimum daily calibration requirements using the internal standard calibration procedure consist of initial analyses of a calibration check standard followed by verification of the internal standard response of each sample applying criterion described in Section 10.4.

9.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from reagents.

10. QUALITY CONTROL

10.1 Each laboratory that uses this method is required to operate a formal quality control (QC) program. The minimum QC requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified blanks, laboratory fortified matrix samples and QC samples. Additional quality control practices are recommended.

10.2 LABORATORY REAGENT BLANKS (LRB) - Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is analyzed or reagents are changed, a LRB must be analyzed. For this method, the LRB is filtered reagent water. If within the retention time window of an analyte of interest, the LRB produces a peak that interferes with analyte determination, determine the source of contamination and eliminate the interference before processing samples.

10.3 INITIAL DEMONSTRATION OF CAPABILITY

10.3.1 Select a representative spike concentration (about 10 times MDL) for each analyte. Prepare a laboratory control sample concentrate (in acetonitrile) from the stock standard solutions containing each analyte at 1000 times the selected concentration. Using a pipet, add 1.00 mL of the concentrate to each of at least four 1 L aliquots of reagent water and analyze each aliquot according to procedures beginning in Section 11.2.

10.3.2 For each analyte, the recovery value must for at least three out of four consecutively analyzed samples fall in the range of R ± 30% (or within R ± 3 S_r, if broader) using the values for R and S_r for reagent water in Table 2 (fortification level 1). The relative standard deviation of the mean recovery measured in 10.3.1 should be ± 30% or $3S_r$ (whichever is greater), using the values of S_r (level 1)) in Table 2. For those compounds that meet the acceptance criteria, performance is acceptable and sample analysis may begin. For those compounds that fail these criteria, initial demonstration of capability must be repeated.

10.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples by a new, unfamiliar method prior to evidencing a basal level of skill at performing the technique. It is expected that as laboratory personnel gain experience with this method the quality of the data will improve beyond the requirements stated in Section 10.3.2.

10.3.4 The analyst is permitted to modify HPLC columns, HPLC conditions, or detectors to improve separations or lower analytical costs. Each time such method modifications are made, the analyst must repeat the procedures in Section 10.3.

10.4 ASSESSING THE INTERNAL STANDARD - When using the IS calibration procedure, the analyst is expected to monitor the IS response (peak area or peak height) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from the daily mean IS response by more than 30%.

10.4.1 If a deviation of > 30% is encountered for a sample, re-inject the extract.

10.4.1.1 If acceptable IS response is achieved for the re-injected extract, then report the results for that sample.

10.4.1.2 If a deviation of > 30% is obtained for the re-injected extract, analysis of the sample should be repeated beginning with Section 11.2, provided the sample is still available. Otherwise, report results obtained from the re-injected extract, but annotate as suspect.

10.4.2 If consecutive samples fail the IS response acceptance criterion, immediately analyze a calibration check standard.

10.4.2.1 If the check standard provides a response factor (RF) within 20% of the predicted value, then follow procedures itemized in Section 10.4.1 for each sample failing the IS response criterion.

10.4.2.2. If the check standard provides a response factor (RF) which deviates more than 20% of the predicted value, then the analyst must recalibrate, as specified in Section 9.3.

10.5 LABORATORY FORTIFIED BLANK

10.5.1 The laboratory must analyze at least one laboratory fortified blank (LFB) per sample set (all samples analyzed within a 24 hour period). The concentration of each analyte in the LFB should be 10 times the MDL. Calculate accuracy as percent recovery (R). If the recovery of any analyte falls outside the control limits (See Section 10.5.2), that analyte is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.

10.5.2 Until sufficient LFB data become available, usually a minimum of results from 20 to 30 analyses, the laboratory must assess its performance against the control limits described in

Section 10.3.2. When sufficient laboratory performance data becomes available, develop control limits from the mean percent recovery (R) and standard deviation (S_r) of the percent recovery. These data are used to establish upper and lower control limits as follows:

Upper Control Limit = R + $3S_r$
Lower Control Limit = R - $3S_r$

After each group of five to ten new recovery measurements, control limits should be recalculated using only the most recent 20 to 30 data points.

10.6 LABORATORY FORTIFIED MATRIX SAMPLE (LFM)

10.6.1 The laboratory must add a known fortified concentration to a minimum of 10% of the routine samples or one fortified sample per set, whichever is greater. The fortified concentration should not be less than the background concentration of the original sample. Ideally, the fortified concentration should be the same as that used for the LFB (Section 10.5). Over time, samples from all routine sample sources should be fortified.

10.6.2 Calculate the percent recovery (R) for each analyte, corrected for background concentrations measured in the original sample, and compare these values to the control limits established in Section 10.5.2 for the analyses of LFBs.

10.6.3 If the recovery of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Section 10.5), the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. The result for that analyte in the original sample must be labelled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

10.7 QUALITY CONTROL SAMPLES (QCS) - Each quarter, the laboratory should analyze one or more QCS (if available). If criteria provided with the QCS are not met, corrective action should be taken and documented.

10.8 The laboratory may adopt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

11. PROCEDURE

11.1 SAMPLE CLEANUP - Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a cleanup procedure, the analyst first must demonstrate that the requirements of Section 10.5 can be met using the method as revised to incorporate the cleanup procedure. EPA Method 610 describes one possible cleanup procedure for this analyte list.

11.2 SAMPLE EXTRACTION - LIQUID-SOLID EXTRACTION (LSE)

11.2.1 Preparation of liquid-solid extraction cartridges.

11.2.1.1 Wash each C_{18} (1.0g) cartridge with four 10 mL aliquots of methylene chloride ($MeCl_2$). Let the cartridge drain after each wash.

11.2.1.2 Wash each cartridge with four 10 mL aliquots of methanol (MeOH), letting the cartridge drain after each wash.

11.2.1.3 Wash the cartridges with two, 10 mL aliquots of reagent water. Allow the first 10 mL portion to wash through letting the cartridge drain dry. Next wash the last 10 mL portion through keeping the cartridge wet. (Water level just above the packing).

11.2.2 Mark the water meniscus on the side of the sample bottle (approximately 1 L) for later determination of sample volume. Pour the entire sample into a 2 L separatory funnel. Add an aliquot of the fortification solution of internal standards (50-100 µg/mL) described in 7.8. The addition of a 100 µL aliquot will yield internal standard concentrations of 5-10 µg/L in aqueous solution. The optimum internal standard concentrations employed will depend upon the UV absorbance and/or fluorescence properties of the compounds. Concentrations should be selected which yield peak area counts equivalent to the upper range of analyte concentrations.

11.2.3 Attach a prepared C_{18} cartridge (Section 11.2.1) on a 1 L vacuum flask. Attach a 75 mL reservoir to the C_{18} cartridge with an appropriate adaptor. Position the 2 L separatory funnel with the sample so that the sample can be run into the 75 mL reservoir. Connect the vacuum source (hose of vacuum pump) to the 1 L vacuum flask and filter the entire sample through the cartridge extraction train. Adjust vacuum to 8-10 mm Hg.

11.2.4 Wash the cartridge with 10 mL of reagent water. Continue to draw vacuum through the cartridge for an additional 10 minutes to dry the cartridge. Release the vacuum and discard the sample waste.

11.2.5 Elute the sample from the cartridge with two 5 mL portions of $MeCl_2$. Wash the 2 L separatory funnel with 2 mL of $MeCl_2$ and add to the cartridge extract. Note: All glass surfaces coming in contact with the aqueous sample must be washed with methylene chloride (1 mL per container) and added to the column eluate.

11.2.6 Prepare a chromatographic column by packing it with 1 inch of anhydrous sodium sulfate. Wet the sodium sulfate by passing 10 mL of methylene chloride through the column. Pour the cartridge extract and washings from Section 11.2.5 through the chromatographic column and collect into a calibrated 10 mL Kuderna-Danish concentrator tube.

11.2.7 Rinse the drying column with an additional 2 mL of $MeCl_2$ and collect in the concentrator tube. Stopper the concentrator tube and store refrigerated (4°C) if further processing will not be performed immediately. If the extract will be stored longer than two days, it should be transferred to a Teflon-sealed screw-cap vial and protected from light.

11.2.8 Evaporate the eluate with a stream of N_2 to a volume of 1.0 mL. Add 3.0 mL of acetonitrile (MeCN) and concentrate to a final volume of 0.5 mL. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract will be stored longer than two days, it should be transferred to a Teflon-sealed screw cap vial and protected from light. If the sample extract requires no further cleanup, proceed with liquid chromatographic analysis (Section 11.3).

11.2.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11.3 SAMPLE ANALYSIS

11.3.1 Table 1 summarizes the recommended operating conditions for the HPLC. Included in this table are retention times and MDLs that can be achieved under these conditions. The UV detector is recommended for the determination of naphthalene, acenaphthylene, acenaphthene and fluorene. The fluorescence detector is recommended for the remaining PAHs. An example for the separation achieved by this HPLC column is shown in Figure 1. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 10.5 are met.

11.3.2 Calibrate the system daily as described in Section 9.

11.3.3 Inject 5 to 100 µL of the sample extract or standard into the HPLC using a high pressure syringe or a constant volume sample injection loop. Record the volume injected to the nearest 0.1 µL, and the resulting peak size in area or peak height units. Re-equilibrate the HPLC column at the initial gradient conditions for at least 10 minutes between injections.

11.3.4 Identify the analytes in the sample by comparing the retention time of the peaks in the sample chromatogram with those of the peaks in standard chromatograms. The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.3.5 Identification requires expert judgement when sample components are not resolved chromatographically, that is, when GC peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima), or any time doubt exists over the identification of a peak in a chromatogram, appropriate confirmatory techniques need to be employed such as use of an alternative detector which operates on a chemical/physical principle different from that originally used, e.g., mass spectrometry, or the use of a second chromatography column.

11.3.6 If the response for a peak exceeds the working range of the system, dilute the extract with acetonitrile and reanalyze.

11.3.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

12. CALCULATIONS

12.1 Determine the concentration of individual compounds in the sample.

12.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor determined in Section 9.2.2. The concentration in the sample can be calculated from Equation 2.

Equation 2

$$\text{Concentration } (\mu g/L) = \frac{(A)\,(V_t)}{(V_i)\,(V_s)}$$

where:

A = Amount of material injected (ng).
Vi = Volume of extract injected (μL).
Vt = Volume of total extract (μL).
Vs = Volume of water extraction (mL).

12.1.2 If the internal standard calibration procedure is used, calculate the concentration in the sample using the response factor (RF) determined in Section 9.3.2 and Equation 3.

Equation 3

$$\text{Concentration } (\mu g/L) = \frac{(A_s)\,(I_s)}{(A_{is})\,(RF)\,(V_o)}$$

where:

As = Response for the parameter to be measured.
Ais = Response for the internal standard.
Is = Amount of internal standard added to each extract (μg).
Vo = Volume of water extracted (L).

12.2 Report results in μg/L without correction for recovery data. All QC data obtained should be reported with the sample results.

13. METHOD PERFORMANCE

13.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL is equal to the level calculated by multiplying the standard deviation of N replicate measurements times the students' t test critical value for a 99 percent confidence level at N - 1 degrees of freedom (1).

13.2 In a single laboratory, analyte recoveries from reagent water were determined at two concentration levels. Results were used to determine analyte MDLs and demonstrate method range. Analytes were divided into three spiking sets: compounds measured by UV detection (UV) and two groups of compounds measured by fluorescence detection (FD-A and FD-B), and analyzed separately. MDL values are given in Table 1. Precision and accuracy data obtained for the two concentration levels in reagent water are presented in Table 2.

13.3 In a single laboratory, analyte recoveries from dechlorinated tap water were determined at one concentration level. Results were used to demonstrate method performance capabilities for a finished drinking water matrix. As with Section 13.2, analytes were grouped into three spiking sets (UV, FD-A and FD-B). Precision and accuracy results for the dechlorinated tap water are shown in Table 3.

14. REFERENCES

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TABLE 1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY CONDITIONS AND METHOD DETECTION LIMITS

Analyte	Sample Set	Retention Time min	Method Detection Limit µg/L (a)	Method Detection Limit Fortification Level µg/L
Naphthalene	UV	12.5	2.20	10.0
Acenaphthylene	UV	13.8	1.41	10.0
Acenaphthene	UV	15.4	2.04	10.0
Fluorene	UV	15.8	0.126	1.00
Phenanthrene	FD-B	16.8	0.150	0.500
Anthracene	FD-A	17.6	0.140	0.625
Fluoranthene	FD-B	18.7	0.009	0.025
Pyrene	FD-A	19.4	0.126	0.625
Benzo(a)anthracene	FD-B	21.9	0.004	0.010
Chrysene	FD-A	22.3	0.160	0.625
Benzo(b)fluoranthene	FD-B	24.2	0.006	0.010
Benzo(k)fluoranthene	FD-A	25.0	0.003	0.0125
Benzo(a)pyrene	FD-B	26.0	0.016	0.050
Dibenzo(a,h)anthracene	FD-B	27.1	0.035	0.125
Benzo(g,h,i)perylene	FD-B	27.8	0.020	0.050
Indeno(1,2,3-cd)pyrene	FD-A	28.3	0.036	0.125

HPLC column conditions: Reverse-phase LC-PAH, 5 micron particle size, in a 25 cm x 4.6 mm ID stainless steel column. Isocratic elution for 2 min. using acetonitrile/water (3.5 : 6.5), then linear gradient elution to 100% acetonitrile over 12 min. at 2.0 mL/min. flow rate.

(a) The MDL for naphthalene, acenaphthylene, acenaphthene, and fluorene were determined using a UV detector. All others were determined using a fluorescence detector.

TABLE 2. SINGLE-LABORATORY ACCURACY AND PRECISION FROM SEVEN REPLICATE ANALYSES OF FORTIFIED REAGENT WATER

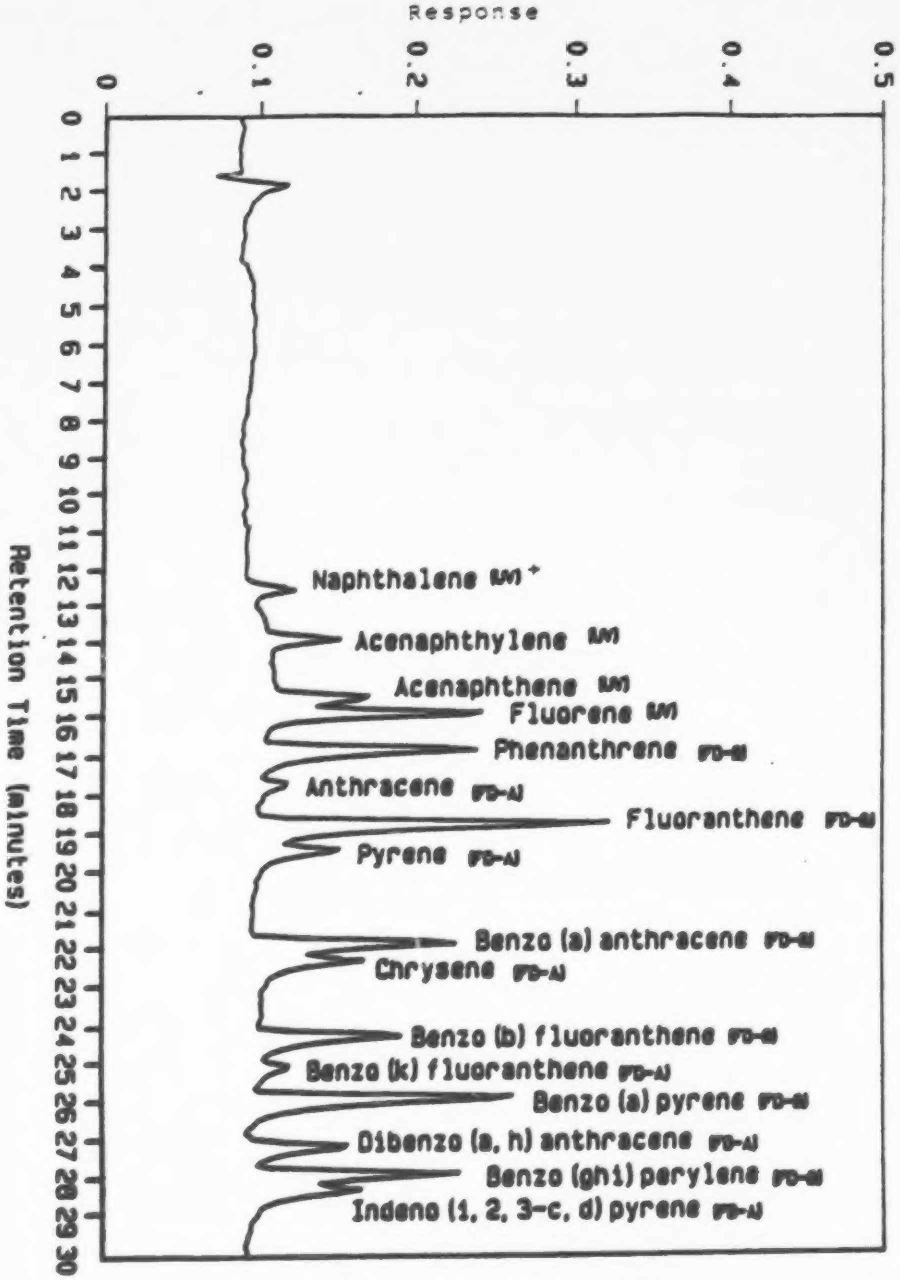
Analyte	Concentration Level 1 µg/L	R(a)	S_r(b)	Concentration Level 2 µg/L	R	S_r
Naphthalene	10.0	70.5	7.0	2.00	85.8	12.8
Acenaphthylene	10.0	78.0	4.5	2.00	63.0	35.0
Acenaphthene	10.0	79.0	6.5	2.00	46.0	9.2
Fluorene	1.0	74.5	4.0	0.200	93.8	6.5
Phenanthrene	0.500	66.9	9.3	0.100	118	57.0
Anthracene	0.625	72.8	7.2	0.125	68.8	8.8
Fluoranthene	0.025	90.2	12.0	0.005	210	90.0
Pyrene	0.625	88.8	6.4	0.125	82.0	8.4
Benzo(a)anthracene	0.010	76.0	14.0	0.002	125	75.0
Chrysene	0.625	93.6	8.0	0.125	81.6	3.6
Benzo(b)fluoranthene	0.010	87.5	18.5	0.002	250	175
Benzo(k)fluoranthene	0.0125	81.2	7.2	0.0025	60	20.0
Benzo(a)pyrene	0.050	76.5	10.3	0.01	75.0	25.0
Dibenzo(a,h)anthracene	0.125	78.4	8.8	0.025	64.0	10.0
Benzo(g,h,i)perylene	0.050	81.5	13.0	0.01	50.0	10.0
Indeno(1,2,3-cd)pyrene	0.125	75.2	9.2	0.025	58.0	8.0

(a) R = Mean Recovery %
(b) S_r = Standard Deviation of the %
(c) Spike Level 2 = Concentration for analytes which yield a signal-to-noise ratio of approximately 10 in the extract (25 µL injection)

TABLE 3. SINGLE-LABORATORY ACCURACY AND PRECISION FROM SEVEN REPLICATE ANALYSES OF FORTIFIED TAP WATER (a)

Analyte	Fortified Concentration Level µg/L	R	S_r
Naphthalene	10.0	72.5	6.5
Acenaphthylene	10.0	78.5	4.5
Acenaphthene	10.0	85.0	5.0
Fluorene	1.0	76.0	3.5
Phenanthrene	0.5	66.2	9.6
Anthracene	0.625	69.5	9.2
Fluoranthene	0.025	66.0	14.0
Pyrene	0.625	72.2	10.2
Benzo(a)anthracene	0.01	20.0	5.0
Chrysene	0.625	80.0	8.6
Benzo(b)fluoranthene	0.01	65.0	5.0
Benzo(k)fluoranthene	0.0125	68.0	8.0
Benzo(a)pyrene	0.05	13.3	11.7
Dibenzo(a,h)anthracene	0.125	77.6	6.4
Benzo(g,h,i)perylene	0.05	24.0	10.0
Indeno(1,2,3-cd)pyrene	0.125	60.8	6.0

(a) Tap water was dechlorinated with sodium thiosulfate (100 mg/L) prior to spiking with analytes.



+ Analysis set (See Section 14)

Figure 1. PAH HPLC Chromatogram using UV Detection. Chromatographic Conditions are as Stated in Table 1.

METHOD 551. THE ANALYSIS OF CHLORINATION DISINFECTION BYPRODUCTS AND CHLORINATED SOLVENTS IN DRINKING WATER BY A LIQUID/LIQUID EXTRACTION PROCEDURE

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METHOD 551

THE ANALYSIS OF CHLORINATION DISINFECTION BYPRODUCTS AND CHLORINATED SOLVENTS IN DRINKING WATER BY A LIQUID/LIQUID EXTRACTION PROCEDURE

1. SCOPE AND APPLICATION

1.1 This method (1-4) is applicable to the determination of the following analytes in finished drinking water, drinking water during intermediate stages of treatment, and raw source water:

ANALYTE	CAS No.
Bromochloroacetonitrile	83463-62-1
Bromodichloromethane	75-27-4
Bromoform	75-25-2
Carbon Tetrachloride	56-23-5
Chloral Hydrate	75-87-6
Chloroform	67-66-3
Chloropicrin	76-06-2
Dibromoacetonitrile	3252-43-5
Dibromochloromethane	124-48-1
1,2-Dibromo-3-chloropropane [DBCP]	96-12-8
1,2-Dibromoethane [EDB]	106-93-4
Dichloroacetonitrile	3018-12-0
Trichloroacetonitrile	545-06-2
Tetrachloroethylene	127-18-4
1,1,1-Trichloroethane	71-55-6
Trichloroethylene	79-01-6
1,1,1-Trichloropropanone -2	918-00-3
1,1-Dichloropropanone -2	513-88-2

1.2 This analyte list includes six commonly used chlorinated organic solvents - carbon tetrachloride, 1,2-dibromo-3-chloropropane (DBCP), 1,2-dibromoethane (EDB), tetrachloroethylene, 1,1,1-trichloroethane and trichloroethylene.

The remaining analytes are organic chlorination byproducts, which are ubiquitously formed (3,4) and observed in chlorinated water supplies over the range of concentrations in Tables 2-5.

1.3 The experimentally determined method detection limits (MDLs) (5) for the above listed analytes are provided in Table 2. Actual MDL values will vary according to the particular matrix analyzed and the specific instrumentation employed.

2. SUMMARY OF METHOD

2.1 A 35 ml sample aliquot is extracted with 2 ml of methyl-tert-butyl ether (MTBE). Two µL of the extract is then injected into a GC equipped with a fused silica capillary column and linearized electron capture detector for separation and analysis. Aqueous calibration standards are also extracted and analyzed in order to compensate for any extraction losses. A typical sample can be extracted and analyzed in 40 to 50 min using the primary column chosen for this method (6.8.2.1). Confirmation of the eluted compounds may be obtained using a dissimilar column (6.8.2.2) or by the use of GC-MS.

3. DEFINITIONS

3.1 Internal standard -- A pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.2 Surrogate analyte -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.

3.3 Laboratory duplicates (LD1 and LD2) -- Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 Field duplicates (FD1 and FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.5 Laboratory reagent blank (LRB) -- An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 Field reagent blank (FRB) -- Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 Laboratory fortified blank (LFB) -- An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.

3.8 Laboratory fortified sample matrix (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.9 Stock standard solution -- A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.10 Primary dilution standard solution -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.11 Calibration standard (CAL) -- a solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.12 Quality control sample (QCS) -- a sample matrix containing method analytes or a solution of method analytes in a water miscible solvent which is used to fortify reagent water or environmental samples. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance with externally prepared test materials.

4. INTERFERENCES

4.1 Impurities contained in the extracting solvent usually account for the majority of the analytical problems. Solvent blanks should be analyzed for each new bottle of solvent before use. An interference-free solvent is a solvent containing no peaks yielding data at $\geq$ MDL (Table 2) and at the retention times of the analytes of interest. Indirect daily checks on the extracting solvent are obtained by monitoring the laboratory reagent blanks (10.2). Whenever an interference is noted in the sample blank, the analyst should analyze another solvent blank. Low level interferences generally can be removed by distillation or column chromatography (2).

4.2 Commercial lots of the MTBE extraction solvent often contain observable amounts of chlorinated solvent impurities, e.g., chloroform, trichloroethylene, carbon tetrachloride. When present, these impurities can normally be removed by a double distillation of the MTBE.

4.3 This liquid/liquid extraction technique efficiently extracts a wide boiling range of non-polar and polar organic components of the sample. Thus, confirmation is quite important, particularly at lower analyte concentrations. A confirmatory column (6.8.2.2) is provided for this purpose. Alternatively, a more powerful technique is confirmation by GC-MS.

5. SAFETY

5.1 The toxicity and carcinogenicity of chemicals used in this method have not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals used in this method. Additional references to laboratory safety are available (6-8) for the information of the analyst.

5.2 The following have been tentatively classified as known or suspected human or mammalian carcinogens:

Chloroform, 1,2-Dibromo-3-chloropropane, 1,2-Dibromoethane.

5.3 The toxicity of the extraction solvent, MTBE, has not been well defined. Susceptible individuals may experience adverse affects upon skin contact or inhalation of vapors. Therefore, protective clothing and gloves should be used and MTBE should be used only in a chemical fume hood or glove box. The same precaution applies to pure standard materials.

6. APPARATUS AND EQUIPMENT

(All specifications in Sections 6 and 7 are suggested. Catalogue numbers are included for illustration only.)

6.1 SAMPLE CONTAINERS - 40 mL screw cap vials [Pierce #13075] or equivalent each equipped with a PTFE-faced silicone septum (Pierce #12722, Fisher TFE-lined #02-883-3F or equivalent). NOTE: Some commercial 40 mL vials do not have adequate volume when salt is added. (See Sect. 11.1.4). Prior to use, wash vials and septa with detergent and rinse with tap water, followed by distilled water. Allow the vials and septa to dry at room temperature, place the vials in an oven and heat to 400°C for 30 min. After removal from the oven allow the vials to cool in an area known to be free of organics.

6.2 VIALS - Autosampler, screw cap with septa, 1.8 ml, Varian #96-000099-00 or equivalent.

6.3 MICRO SYRINGES - 10 µL, 25 µL, 50 µL, 100 µL, 250 µL, Hamilton 702N Series or equivalent.

6.4 PIPETTES - 2.0 ml transfer, glass.

6.5 VOLUMETRIC FLASK - 10 ml, 100 ml, 250 ml glass stoppered.

6.6 DISPOSABLE PASTEUR PIPETS - Kimble No. 72050575 or equivalent.

6.7 STANDARD SOLUTION STORAGE CONTAINERS - 15 ml Boston round, amber glass bottles with TFE-lined caps. Wheaton Cat. No. 220092 or equivalent. TFE-lined caps must be purchased separately. Size 18-400, Fisher TFE-lined screw cap No. 02-883-3D or equivalent.

6.8 GAS CHROMATOGRAPHY SYSTEM

6.8.1 The GC must be capable of temperature programming and should be equipped with a linearized electron capture detector, fused silica capillary column, and splitless injector (splitless mode, 30 sec. delay). An auto-sampler/injector is desirable, (e.g., Hewlett Packard 7673A or equivalent).

6.8.2 Two GC columns are recommended. Column A should be used as the primary analytical column unless routinely occurring analytes are not adequately resolved. Column B is recommended for use as a confirmatory column when GC/MS confirmation is unavailable.

6.8.2.1 Column A - 0.32 mm ID x 30 m fused silica capillary with chemically bonded methyl polysiloxane phase (DB-1, 1.0 um film thickness or equivalent). The linear velocity of the helium carrier is established at 23 cm/sec at 35°C. The oven is programmed to hold at 35°C for 9 min, to increase to 40°C at 1°C/min, and held for 3 min, to increase to 120°C at 6°C/min and held at 120°C until all expected compounds have eluted. A temperature of 150°C is then maintained for 5 min. Injector temperature: 200°C. Detector temperature: 290°C (See Table 1 for retention time data).

6.8.2.2 Column B - 0.32 mm ID x 30 m with chemically bonded 50% trifluoropropyl phase (DB-210, SP-2401, 0.5 um film thickness or equivalent). The linear velocity of the helium carrier gas is established at 27 cm/sec. The column temperature is programmed to hold at 30°C for 11 min, to increase to 120°C at 10°C/min and held at 120°C until all expected compounds have eluted. A temperature of 150°C is then maintained for 5 min. (See Table 1 for retention data).

6.9 pH Meter - capable of accurate measurement of pH (± 0.2 units) in the range, pH = 4-8. For laboratory or field measurement of sample pH.

6.10 pH Paper - narrow ranges, pH = 3-5.5 and pH = 6.0-8.0. Fisher catalogue numbers 14-853-70 and 14-853-90, respectively. For measurement of initial and adjusted sample pH in the field.

7. REAGENTS AND CONSUMABLE MATERIALS

7.1 REAGENTS

7.1.1 MTBE - High purity grade, EM Science Omnisolve grade or equivalent. It may be necessary to double distill the solvent if impurities are observed which coelute with some of the more volatile compounds.

7.1.2 Acetone - High purity, Burdick and Jackson #010 or equivalent demonstrated to be free of analytes.

7.1.3 Sodium Chloride, NaCl - ACS Reagent Grade. Before use pulverize a batch of NaCl and place in muffle furnace, increase temperature to 400°C and hold for 30 min. Store in a capped bottle.

7.2 REAGENT WATER - Reagent water is defined as purified water which does not contain any measurable quantities of the analyte or any other interfering species.

7.2.1 A Millipore Super-Q water system or its equivalent may be used to generate deionized reagent water. Distilled water that has been charcoal filtered may also be suitable.

7.2.2 Test reagent water each day it is used by analyzing according to Sect. 10.2.

7.3 STOCK STANDARD SOLUTIONS - These solutions may be obtained as certified solutions or prepared from neat materials using the following procedures:

7.3.1 Prepare stock standard solutions (5.0 mg/ml) by accurately weighing about 0.0500g of pure material. Dissolve the material in acetone and dilute to volume in a 10-ml volumetric flask. Methanol was not used because dihaloacetonitriles, chloropicrin and 1,1,1-trichloropropanone were found to be unstable in methanol. The trihalomethanes (THMs) and the six solvents listed in Sect. 1.1 (EDB, DBCP, carbon tetrachloride, 1,1,1-trichloroethane, trichloroethylene and tetrachloroethylene) may be prepared in methanol. Larger volumes can be used at the discretion of the analyst. When compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

7.3.2 Transfer the stock standard solutions into Teflon-lined screw cap amber bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

7.3.3 The stored THM stock standards in methanol are stable for up to six months. The solvent standards in methanol are stable at least four weeks. The other analytes stored in acetone are stable for at least four months except for chloral hydrate. Initially, fresh chloral hydrate standards should be prepared weekly until the stability of this analyte is determined.

7.4 PRIMARY DILUTION STANDARDS--Use stock standard solutions to prepare dilution standard solutions that contain the analytes in acetone. The primary dilution standards should be prepared at concentrations that can be easily diluted to prepare aqueous calibration standards (Sect. 9.1) that will bracket the working concentration range. Store the primary dilution standard solutions at 4°C with minimal headspace and check frequently for signs of deterioration or evaporation, especially just before preparing calibration standards. The same comments on storage stability in 7.3.3 apply to primary dilution standards.

7.5 Hydrochloric Acid Solutions, 0.2 and 1.0 N - Prepare solutions for adjustment of sample pH by serial dilution of ACS reagent grade hydrochloric acid (HCl).

7.6 Stock Solution of Internal Standard(s) - Prepare a solution of internal standard(s) in methanol at concentration(s) of 0.5-1.0 mg/mL. Dilute an aliquot of the solution with methanol by an appropriate factor (e.g. 1:100) required for the internal standard fortification solution used in preparing calibration standards (9.1.2.2) or fortifying aqueous samples (11.1.3).

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE COLLECTION, DECHLORINATION, AND PRESERVATION

8.1.1 Adjustment of Sample pH - Prior to sample collection, the amount of HCl required to reduce the sample pH to the range, 4.5-5.0 must be measured. Collect 40 mL samples and add to 100 mL beakers containing 10 mg ammonium chloride. Measure the initial pH with the narrow range pH paper, 6.0-8.0 (6.10), or a pH meter. Initially, adjust the sample pH to the range 4.5-5.0 with the 0.2 HCl solution by dropwise addition with a Pasteur pipet (6.6). Measure the pH during addition with the narrow range pH paper, 3.0-5.5, or a pH meter. If greater than 10 drops are required (ca. 0.1 mL), measure the amount of 1.0 N HCl solution required and use this amount for sample acidification in 8.1.2. Care should be exercised not to adjust the sample pH below the carbonic acid endpoint, pH ≈ 4.2. Below the endpoint, the pH will decrease rapidly with dropwise acid addition. Some of the analytes may not be stable below pH = 4.0.

8.1.2 Collect all samples in duplicate. The 40 mL sample vials (6.1) should contain approximately 10 mg of ammonium chloride for dechlorination, prior to transport to the field. Before filling the vial, add the amount of HCl solution determined in 8.1.1 for preservation. Fill sample bottles to just overflowing but take care not to flush out the dechlorination and preservation reagents. No air bubbles should pass through the sample as the bottle is filled, or be trapped in the sample when the bottle is sealed.

8.1.3 When sampling from a water tap, open the tap and allow the system to flush until the water temperature has stabilized (usually about 10 min). Adjust the flow to about 500 mL/min and collect duplicate samples from the flowing stream.

8.1.4 When sampling from an open body of water, fill a 1-quart wide-mouth bottle or 1-liter beaker with sample from a representative area, and carefully fill duplicate sample vials from the 1-quart container.

8.1.5 The samples must be chilled to 4°C on the day of collection and maintained at that temperature until analysis. Field samples that will not be received at the laboratory on the day of collection must be packaged for shipment with sufficient ice to ensure that they will be at 4°C on arrival at the laboratory.

8.1.6 In some matrices dechlorinated with ammonium chloride, fortified matrix recoveries of chloral hydrate have been lower than expected by 50 percent or greater, when compared to the same sample dechlorinated with ascorbic acid. In other matrices, recoveries have been normal. The reason for these differences has not been determined. Any analyst employing this method must demonstrate that ammonium chloride is a suitable dechlorination agent for chloral hydrate in the matrix of concern by determining matrix recoveries as outlined in 10.6. If problems are encountered, a separate sample, dechlorinated with 100 mg/L of ascorbic acid, must be collected for the analysis of chloral hydrate.

8.2 SAMPLE STORAGE

8.2.1 Store samples at 4°C until analysis. The sample storage area must be free of organic solvent vapors.

8.2.2 Analyze all samples within 14 days of collection. Samples not analyzed within this period must be discarded and replaced.

9. CALIBRATION

9.1 PREPARATION OF CALIBRATION STANDARDS

9.1.1 At least three calibration standards are needed. One should contain the analytes at a concentration near to but greater than the method detection limit (Table 2) for each compound; the other two should bracket the concentration range expected in samples. For example, if the MDL is 0.1 µg/L, and a sample expected to contain approximately 1.0 µg/L is to be analyzed, aqueous standards should be prepared at concentrations of 0.2 µg/L, 1.0 µg/L, and 2.0 µg/L.

9.1.2 To prepare a calibration standard, add an appropriate volume of a primary dilution standard to a 35-mL aliquot of reagent water in a 40-mL vial. Use a 25-µL micro syringe and rapidly inject the standard into the middle point of the water volume. Remove the needle as quickly as possible after injection. If required (9.3), add an appropriate volume of the internal standard fortification solution (7.6) in the same manner. The aqueous concentration of internal standard(s) should yield area counts or peak heights equivalent to the medium to upper ranges of analyte concentrations. Mix by inverting the sample vial three times without shaking. Aqueous standards must be prepared fresh daily and extracted immediately after preparation (Section 11).

9.1.3 Alternatively, add an appropriate volume of primary dilution standard and internal standard solution to reagent water in a 100 ml volumetric flask and fill to the mark. Mix by inverting three times as in 9.1.2.1. Weigh a 35 ml aliquot of this standard into a pre-calibrated 40-ml vial.

9.2 EXTERNAL STANDARD CALIBRATION PROCEDURE

9.2.1 Extract and analyze each calibration standard according to Section 11 and tabulate peak height or area response versus the concentration of the standard. The results are used to prepare a calibration curve for each compound by plotting the peak height or area response versus the concentration. Alternatively, if the ratio of response to concentration (response factor) is constant over the working range ($\leq$ 10% relative standard deviation,[RSD]), linearity to the origin can be assumed, and the average ratio or response factor can be used in place of a calibration curve.

9.2.2 Single point calibration is sometimes an acceptable alternative to a calibration curve. Prepare single point standards from the primary dilution standard solutions. The single point calibration standard should be prepared at a concentration that produces a response close (± 20%) to that of the unknowns.

9.3 INTERNAL STANDARD (IS) CALIBRATION PROCEDURE - To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, specific internal standard are not recommended in this method. The method validation data reported in Section 13 were obtained by the external calibration procedure.

9.3.1 Extract and analyze each calibration standard according to Section 11. Tabulate peak height or area responses against concentration for each compound and internal standard. Calculate response factor (RF) for each compound using Equation 1.

Equation 1

$$RF = \frac{[As]\,[Cis]}{[Ais]\,[Cs]}$$

where

As = Response for the analyte to be measured
Ais = Response for the internal standard
Cis = Concentration of the internal standard (µg/L)
Cs = Concentration of the analyte to be measured (µg/L)

If RF value over the working range is constant (< 10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response versus analyte ratios, As/Ais vs. Cs/Cis.

9.4 The working calibration curve, calibration factor, or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any analyte varies from the predicted response by more than ± 20%, the test must be repeated using fresh calibration standard. If the fresh calibration standard also deviates by more ± 20%, a new calibration curve must be prepared for that compound.

9.4.1 Daily calibration requirements using the external standard calibration procedure are a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (> 8 hrs), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analysis.

9.4.2 Minimum daily calibration requirements using the internal standard calibration procedure consist of initial analyses of a calibration check standard followed by verification of the internal standard response of each sample applying criterion described in Section 10.4.

10. QUALITY CONTROL

10.1 Each laboratory that uses this method is required to operate a formal quality control (QC) program. Minimum QC requirements are initial demonstration of laboratory capability, monitoring internal standard peak area or height in each sample and blank, analysis of laboratory reagent blanks, laboratory fortified blanks, laboratory fortified sample matrices, and QC samples. Additional quality control practices are recommended.

10.2 LABORATORY REAGENT BLANKS (LRB). Before processing any samples, the analyst must analyze at least one LRB to demonstrate that all glassware and reagent interferences are under control. In addition, each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window of any analyte (11.3.5), the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

10.3 INITIAL DEMONSTRATION OF CAPABILITY

10.3.1 Select a representative fortified concentration for each of the target analytes. Concentrations near analyte levels in Table 4 are recommended. Prepare a laboratory control (LC) sample concentrate in acetone or methanol 1000 times more concentrated than the selected concentration. With a syringe, add 100 μL of the LC sample concentrate to each of four to seven 100 mL aliquots of reagent water. Analyze the aliquots according to the method beginning in Section 11.

10.3.2 Calculate the mean percent recovery (R) and the standard deviation of the recovery (S_r). For each analyte, the mean recovery values must fall in the range of R ± 30% (or within R ± $3S_r$ if broader) using the values for R and S_r for reagent water in Table 3. The standard deviation should be less than ± 30% or $3S_r$, whichever is larger. For those compounds that meet these criteria, performance is considered acceptable and sample analysis may begin. For those compounds that fail these criteria, this procedure must be repeated using a minimum of five fresh samples until satisfactory performance has been demonstrated.

10.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. It is expected that as laboratory personnel gain experience with this method, the quality of data will improve beyond those required here.

10.3.4 The analyst is permitted to modify GC columns, GC conditions, detectors, extraction techniques, concentration techniques (i.e., evaporation techniques), internal standard or surrogate compounds. Each time such method modifications are made, the analyst must repeat the procedures in Sect. 10.3.1.

10.4 ASSESSING THE INTERNAL STANDARD

10.4.1 When using the internal standard calibration procedure, the analyst is expected to monitor the IS response (peak area or peak height) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from daily calibration standard's IS response by more than 30%.

10.4.2 If >30% deviation occurs with an individual extract, optimize instrument performance and inject a second aliquot of that extract.

10.4.2.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.

10.4.2.2 If a deviation of greater than 30% is obtained for the reinjected extract, analysis of the samples should be repeated beginning with Sect. 11, provided the sample is still available. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

10.4.3 If consecutive samples fail the IS response acceptance criterion, immediately analyze a calibration check standard.

10.4.3.1 If the check standard provides a response factor (RF) within 20% of the predicted value, then follow procedures itemized in Sect. 10.4.2 for each sample failing the IS response criterion.

10.4.3.2 If the check standard provides a response factor which deviates more than 20% of the predicted value, then the analyst must recalibrate, as specified in Sect. 9.

10.5 LABORATORY FORTIFIED BLANK

10.5.1 The laboratory must analyze at least one laboratory fortified blank (LFB) sample with every 20 samples or one per sample set (all samples extracted within a 24-hr period), whichever is greater. Fortified concentrations near those in Table 4 are recommended. Calculate accuracy as percent recovery (R). If the recovery of any analyte falls outside the control limits (see Sect. 10.5.2), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

10.5.2 Prepare control charts based on mean upper and lower control limits, R ± 3 S_r. The initial demonstration of capability (10.3) establishes the initial limits. After each four to six new recovery measurements, recalculate R and S_r using all the data, and construct new control limits. When the total number of data points reach 20, update the control limits by calculating R and S_r using only the most recent 20 data points. At least quarterly, replicates of LFBs should be analyzed to determine the precision of the laboratory measurements. Add these results to the ongoing control charts to document data quality.

10.6 LABORATORY FORTIFIED SAMPLE MATRIX

10.6.1 The laboratory must add known concentrations of analytes to a minimum of 10% of the routine samples or one concentration per sample set, whichever is greater. The concentrations should be equal to or greater than the background concentrations in the sample selected for fortification. Ideally, the concentration should be the same as that used for the laboratory fortified blank (Sect. 10.5). Over time, samples from all routine sample sources should be fortified.

10.6.2 Calculate the mean percent recovery, R, of the concentration for each analyte, after correcting the total mean measured concentration, A, from the fortified sample for the background concentration, B, measured in the unfortified sample, i.e.:

$$R = 100 (A - B) / C,$$

where C is the fortifying concentration. Compare these values to control limits appropriate for reagent water data collected in the same fashion (10.5).

10.6.3 If the analysis of the unfortified sample reveals the absence of measurable background concentrations, and the added concentrations are those specified in Sect. 10.5, then the appropriate control limits would be the acceptance limits in Sect. 10.5.

10.6.4 If the sample contains measurable background concentrations of analytes, calculate mean recovery of the fortified concentration, R, for each such analyte after correcting for the background concentration.

$$R = 100\,(A - B)/C$$

Compare these values to reagent water recovery data, R*, at comparable fortified concentrations from Tables 3-5. Results are considered comparable if the measured recoveries fall within the range,

$$R^* \pm 3S_c,$$

where S_c is the estimated percent relative standard deviation in the measurement of the fortified concentration. By contrast to the measurement of recoveries in reagent water (10.5.2) or matrix samples without background (10.6.3), the relative standard deviation, S_c, must be expressed as the statistical sum of variation from two sources, the measurement of the total concentration as well as the measurement of background concentration. In this case, variances defined as S^2, are additive and S_c can be expressed as

$$S_c^2 = S_a^2 + S_b^2$$

$$\text{or} \quad S_c = (S_a^2 + S_b^2)^{1/2},$$

where S_a and S_b are the percent relative standard deviations of the total measured concentration and the background concentration respectively. The value of S_a may be estimated from the mean measurement of A above or from data at comparable concentrations from Tables 3-5. Likewise, S_b can be measured from repetitive measurements of the background concentration or estimated from comparable concentration data from Tables 2-5.

10.6.5 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Sect. 10.5), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

10.7 QUALITY CONTROL SAMPLE (QCS) - At least quarterly, analyze a QCS from an external source. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem source.

10.8 The laboratory may adapt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

11. PROCEDURE

11.1 SAMPLE PREPARATION

11.1.1 Remove samples from storage and allow them to equilibrate to room temperature.

11.1.2 Remove the vial caps. Discard a 5-ml volume of the sample. Replace the vial caps and weigh the containers with contents to the nearest 0.1 g and record these weights for subsequent sample volume determination. (See Sect. 11.2.4 for continuation of weighing and calculation of true volume). Alternatively, the sample vials may be precalibrated by weighing in 35 ml of water and scoring the meniscus on the bottle. This will eliminate the gravimetric step above and in 11.2.4.

11.1.3 Inject an aliquot of the internal standard fortification solution (7.6) into the sample. The aqueous concentration of internal standard(s) must be the same as that used in preparing calibration standards (9.1.2).

11.1.4 Remove the vial cap of each sample and add 8 g NaCl (Sect. 7.1.3) to the sample vial. Recap and dissolve the NaCl by inverting and shaking the vial vigorously (approx. 20 sec.).

11.2 SAMPLE EXTRACTION

11.2.1 Remove the vial cap and add 2 mL of MTBE with a transfer or automatic dispensing pipet. Recap and shake by hand for 1 min. Invert the vial and allow the water and MTBE phases to separate (approx. 2 min).

11.2.2 By using a disposable Pasteur pipet (6.6), transfer a portion of the solvent phase from the 40 mL vial to an autosampler vial. Be certain no water has carried over onto the bottom of the autosampler vial. If a dual phase appears in the autosampler vial, the bottom layer can be easily removed and discarded by using a Pasteur pipet. The remaining MTBE phase may be transferred to a second autosampler vial for a subsequent analysis. Approximately 1.5 mL of the solvent phase can be conveniently transferred from the original 2 mL volume.

11.2.3 Discard the remaining contents of the sample vial. Shake off the last few drops with short, brisk wrist movements.

11.2.4 Reweigh the empty vial with the original cap and calculate the net weight of sample by difference to the nearest 0.1 g (Sect. 11.1.2 minus Sect. 11.2.4). This net weight (in grams) is equivalent to the volume of water (in mL) extracted, V_s.

11.3 SAMPLE ANALYSIS AND IDENTIFICATION

11.3.1 The recommended GC operating conditions are described in 6.8.2.1 and 6.8.2.2 along with recommended primary and confirmation columns. Retention data for the primary and confirmation columns are given in Table 1 and examples of separations attained with the primary column are shown in Figures 1 and 2. Other GC columns, chromatographic conditions, or detectors may be used if the requirements of Section 10 are met.

11.3.2 Calibrate the system daily as described in Section 9. The standards and extracts must be in MTBE.

11.3.3 Inject 1-2 µL of the sample extract and record the resulting peak size in area units. For optimum performance and precision, an autosampler for sample injection and a data system for signal processing are strongly recommended.

11.3.4 Identify sample components by comparison of retention times to retention data from a reference chromatogram. If the retention time of an unknown compound corresponds, within limits (11.3.5), to the retention time of a standard compound, then identification is considered positive.

11.3.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.3.6 Identification requires expert judgment when sample components are not resolved chromatographically, that is, when GC peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima). Whenever doubt exists over the identification of a peak in a chromatogram, confirmation is required by the use of a dissimilar column or by GC-MS.

11.3.7 If the peak area exceeds the linear range of the calibration curve, the final extract should be diluted with MTBE and reanalyzed.

12. CALCULATIONS

12.1 Calculate the uncorrected concentrations (Ci) of each analyte in the sample from the response factors or calibration curves generated in 9.2.1 or 9.3.1.

12.2 Calculate the corrected sample concentration as:

$$\text{Concentration, } \mu g/L = Ci \times \frac{35}{Vs},$$

where the sample volume, Vs in mL, is equivalent to the net sample weight in grams determined in 11.1.2 and 11.2.4.

13. METHOD PERFORMANCE

13.1 Single laboratory (EMSL- Cincinnati) recovery and precision data at three concentrations in a reagent water matrix are presented in Tables 3-5. Recovery values were calculated on the basis of unextracted standards. Accuracy and precision data based on extracted standards for fortified tap water, raw source water and groundwater are presented in Tables 6-8.

14. REFERENCES

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TABLE 1. RETENTION DATA

	Column A	Column B
Analyte	Retention Time (min)	Time (min)
Chloroform	5.25	3.09
1,1,1-Trichloroethane	6.37	2.04
Carbon Tetrachloride	7.29	3.41
Trichloroacetonitrile	7.59	5.03
Dichloroacetonitrile	8.72	9.09
Bromodichloromethane	9.02	4.21
Trichloroethylene	9.13	4.38
Chloral Hydrate	9.70	6.56
1,1,-dichloropropanone-2	10.73	11.19
Chloropicrin	15.8	39.94
Dibromochloromethane	16.40	6.40
Bromochloroacetonitrile	16.77	14.43
1,2-Dibromoethane (EDB)	17.40	9.71
Tetrachloroethylene	19.57	6.94
1,1,1-Trichloropropanone	21.36	15.66
Bromoform	23.54	10.73
Dibromoacetonitrile	24.03	17.45
1,2-Dibromo-3-Chloropropane (DBCP)	32.32	20.35

Column A: DB-1, 0.32 mm x 30 m, 1 micron film thickness
Column B: DB-210, 0.32 mm x 30 m, 0.5 micron film thickness

TABLE 2. METHOD DETECTION LIMITS

Analyte	Fortified Conc. µg/L	Mean Meas. Conc. µg/L	Std. Dev. µg/L	Rel. Std. Dev., %	Method Detection Limit µg/L
Bromochloroacetonitrile	.029	.027	.0030	11.1	.011
Bromodichloromethane	.023	.023	.0018	7.9	.006
Bromoform	.029	.028	.0035	12.7	.012
Carbon Tetrachloride	.020	.019	.0014	7.2	.004
Chloral Hydrate	.035	.037	.0091	24.8	.026
Chloroform	.005	.005	.00063	12.7	.002
Chloropicrin	.029	.030	.0033	11.2	.012
Dibromoacetonitrile	.080	.072	.0097	13.5	.034
Dibromochloromethane	.023	.023	.0034	14.8	.012
1,2-Dibromoethane	.028	.028	.0017	6.0	.006
1,2-Dibromo-3-chloropropane	.043	.041	.0027	6.6	.009
Dichloroacetonitrile	.032	.036	.0063	17.6	.019
1,1-Dichloropropanone-2	.021	.020	.0013	6.7	.005
Tetrachloroethylene	.022	.022	.0013	5.9	.004
Trichloroacetonitrile	.143	.135	.0307	22.7	.092
1,1,1-Trichloroethane	.032	.033	.0025	7.7	.008
Trichloroethylene	.018	.023	.00076	3.4	.002
1,1,1-Trichloropropanone	.043	.044	.0033	7.6	.012

TABLE 3. ANALYTE RECOVERY AND PRECISION DATA FROM SEVEN ANALYSES OF FORTIFIED REAGENT WATER

ANALYTE	True Conc. ug/L	Mean Measured Conc. ug/L	Relative Accuracy (Recovery) %	Relative Standard Deviation %
Bromochloroacetonitrile	0.14	0.134	96	3.7
Bromodichloromethane	0.86	0.852	99	2.7
Bromoform	0.20	0.218	109	0.7
Chloral Hydrate	1.60	1.03	64	4.6
Chloroform	2.00	1.60	80	2.4
Chloropicrin	0.16	0.132	83	3.8
Dibromoacetonitrile	0.14	0.134	96	5.8
Dibromochloromethane	0.77	0.804	104	1.5
Dichloroacetonitrile	0.21	0.185	88	3.1
1,1-Dichloropropanone-2	0.10	0.082	82	3.0
Trichloroacetonitrile	0.25	0.179	72	5.3
1,1,1-Trichloropropanone-2	0.18	0.150	83	2.8

TABLE 4. ANALYTE RECOVERY AND PRECISION DATA FROM SEVEN ANALYSES OF FORTIFIED REAGENT WATER

ANALYTE	True Conc. ug/L	Mean Measured Conc. ug/L	Relative Accuracy (Recovery) %	Relative Standard Deviation %
Bromochloroacetonitrile	1.5	1.67	111	2.7
Bromodichloromethane	11.6	12.4	107	2.7
Bromoform	1.9	2.19	115	0.9
Carbon Tetrachloride	2.3	1.98	86	6.9
Chloral Hydrate	23.4	17.1	73	1.6
Chloroform	15.0	15.5	103	3.2
Chloropicrin	1.8	1.62	90	8.9
Dibromoacetonitrile	1.5	1.62	108	5.6
Dibromochloromethane	10.7	12.2	114	2.0
1,2-Dibromoethane	5.0	5.15	103	4.8
1,2-Dibromo-3-chloropropane	5.0	5.60	112	4.7
Dichloroacetonitrile	2.7	2.56	95	1.5
1,1-Dichloropropanone-2	0.92	0.74	80	1.7
Tetrachloroethylene	2.3	1.99	87	6.6
Trichloroacetonitrile	3.6	2.50	69	6.8
1,1,1-Trichloroethane	2.3	2.18	95	8.3
Trichloroethylene	2.3	2.07	90	10
1,1,1-Trichloropropanone-2	2.3	1.95	85	1.9

TABLE 5. ANALYTE RECOVERY AND PRECISION DATA FROM SEVEN ANALYSES OF FORTIFIED REAGENT WATER

ANALYTE	True Conc. ug/L	Mean Measured Conc. ug/L	Relative Accuracy (Recovery) %	Relative Standard Deviation %
Bromochloroacetonitrile	7.7	7.83	102	5.5
Bromodichloromethane	27	27.0	100	5.4
Bromoform	7.6	7.99	105	1.4
Carbon Tetrachloride	11	9.13	83	13
Chloral Hydrate	42	36.1	86	4.6
Chloroform	54	49.4	91	1.5
Chloropicrin	10	8.37	84	11
Dibromoacetonitrile	7.9	7.60	96	7.9
Dibromochloromethane	42	42.2	100	3.5
1,2-Dibromoethane	10	10.1	101	7.5
1,2-Dibromo-3-chloropropane	10	11.3	113	7.5
Dichloroacetonitrile	11	9.7	88	4.5
1,1-Dichloropropanone-2	4.3	3.19	74	2.7
Tetrachloroethylene	11	9.46	86	12
Trichloroacetonitrile	16	12.1	76	3.7
1,1,1-Trichloroethane	11	8.80	80	11
Trichloroethylene	11	9.24	84	11
1,1,1-Trichloropropanone-2	11	8.5	77	1.6

TABLE 6. ANALYTE RECOVERY AND PRECISION DATA FROM SEVEN ANALYSES OF FORTIFIED TAP WATER

ANALYTE	Back-ground Conc. ug/L	Fortified Conc. ug/L	Total Measured Conc. ug/L	Net Conc. ug/L	Accuracy %	Relative Standard Deviation %
Bromochloroacetonitrile	1.26	4.0	6.31	5.05	126	2.8
Bromodichloromethane	12.5	20.0	23.5	11.0	55	5.2
Bromoform	5.83	8.0	13.41	7.58	95	2.5
Chloral Hydrate	2.72	10.0	9.09	6.37	44	7.2
Chloroform	19.4	15.0	30.3	10.9	70	2.2
Chloropicrin	0.26	4.0	5.23	4.97	124	2.6
Dibromoacetonitrile	2.18	6.0	10.35	8.17	136	1.6
Dibromochloromethane	8.69	20	30.9	22.2	111	3.8
Dichloroacetonitrile	5.35	6.0	11.90	6.55	109	2.7
1,1-Dichloropropanone-2	0.43	2.0	2.36	1.93	98	5.3
Trichloroacetonitrile	0.02	4.0	4.10	4.08	102	3.8
1,1,1-Trichloropropanone-2	1.61	4.0	5.82	4.21	105	3.0

TABLE 7. ANALYTE RECOVERY AND PRECISION DATA FROM SEVEN ANALYSES OF FORTIFIED RAW SOURCE WATER

ANALYTE	Back-ground Conc. ug/L	Fortified Conc. ug/L	Total Measured Conc. ug/L	Net Conc. ug/L	Accuracy %	Relative Standard Deviation %
Bromochloroacetonitrile		4.0	3.67	3.67	92	4.2
Bromodichloromethane	0.06	20.0	20.03	19.97	100	2.1
Bromoform	0.13	8.0	9.14	9.01	111	1.7
Chloral Hydrate		10.0	11.72	11.72	117	1.6
Chloroform	2.08	15.0	19.34	17.26	109	2.7
Chloropicrin		4.0	4.60	4.60	116	4.8
Dibromoacetonitrile		6.0	5.66	5.66	94	4.8
Dibromochloromethane	0.04	20.0	23.18	23.14	111	2.2
Dichloroacetonitrile	0.04	6.0	6.28	6.24	103	1.5
1,1-Dichloropropanone-2		2.0	1.88	1.88	94	1.3
Trichloroacetonitrile		4.0	4.55	4.55	114	6.9
1,1,1-Trichloropropanone-2	0.03	4.0	3.96	3.93	98	2.2

TABLE 8. ANALYTE RECOVERY AND PRECISION DATA FROM SEVEN ANALYSES OF FORTIFIED GROUND WATER

ANALYTE	Fortified Conc. ug/L	Mean Measured Conc. ug/L	Accuracy %	Relative Standard Deviation %
Bromochloroacetonitrile	2.0	1.55	78	4.8
Bromodichloromethane	12	13.2	110	1.9
Bromoform	2.0	2.03	102	1.9
Chloral Hydrate	12	14.3	119	5.5
Chloroform	16	18.6	116	1.3
Chloropicrin	2.0	1.29	65	7.7
Dibromoacetonitrile	2.0	1.53	77	1.0
Dibromochloromethane	5.0	4.75	95	3.0
Dichloroacetonitrile	3.0	2.68	89	1.8
1,1-Dichloropropanone-2	1.0	0.90	90	2.1
Trichloroacetonitrile	4.0	5.58	140	2.6
1,1,1-Trichloropropanone-2	2.0	1.82	91	2.5

FIGURE 1

Chlorination Byproducts - DB-1 Primary Column

	Concentration (μg/L)
1. Chloroform	13.8
2. TCAN	10.8
3. DCAN	2.4
4. BDCM	2.4
5. MTBE Contaminant	---
6. CH	17.5
7. DCP	---
8. CP	10.0
9. DBCM	3.2
10. BCAN	9.9
11. EDB	4.7
12. TCP	18.7
13. Bromoform	11.9
14. DBAN	5.3
15. DBCP	2.2

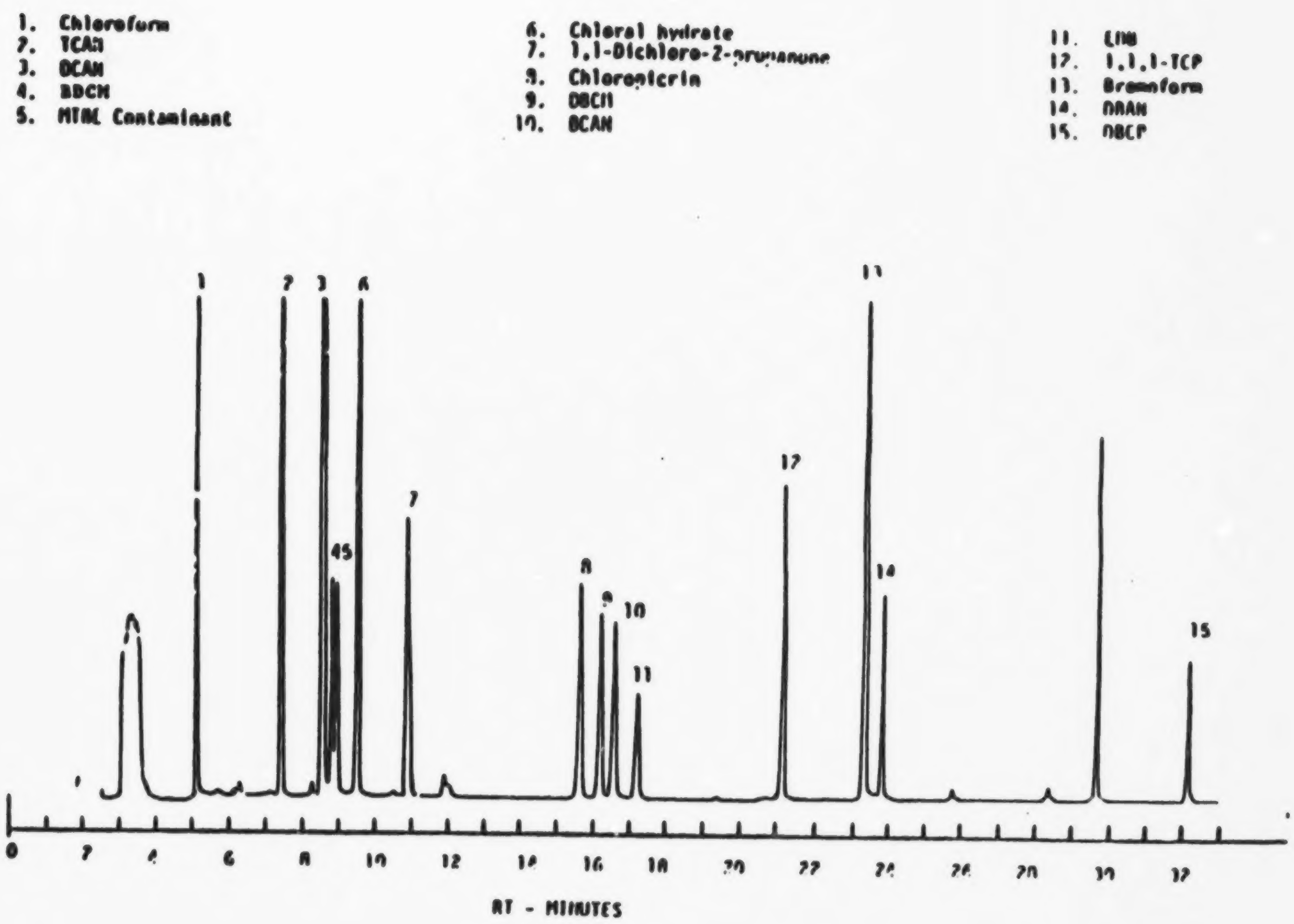
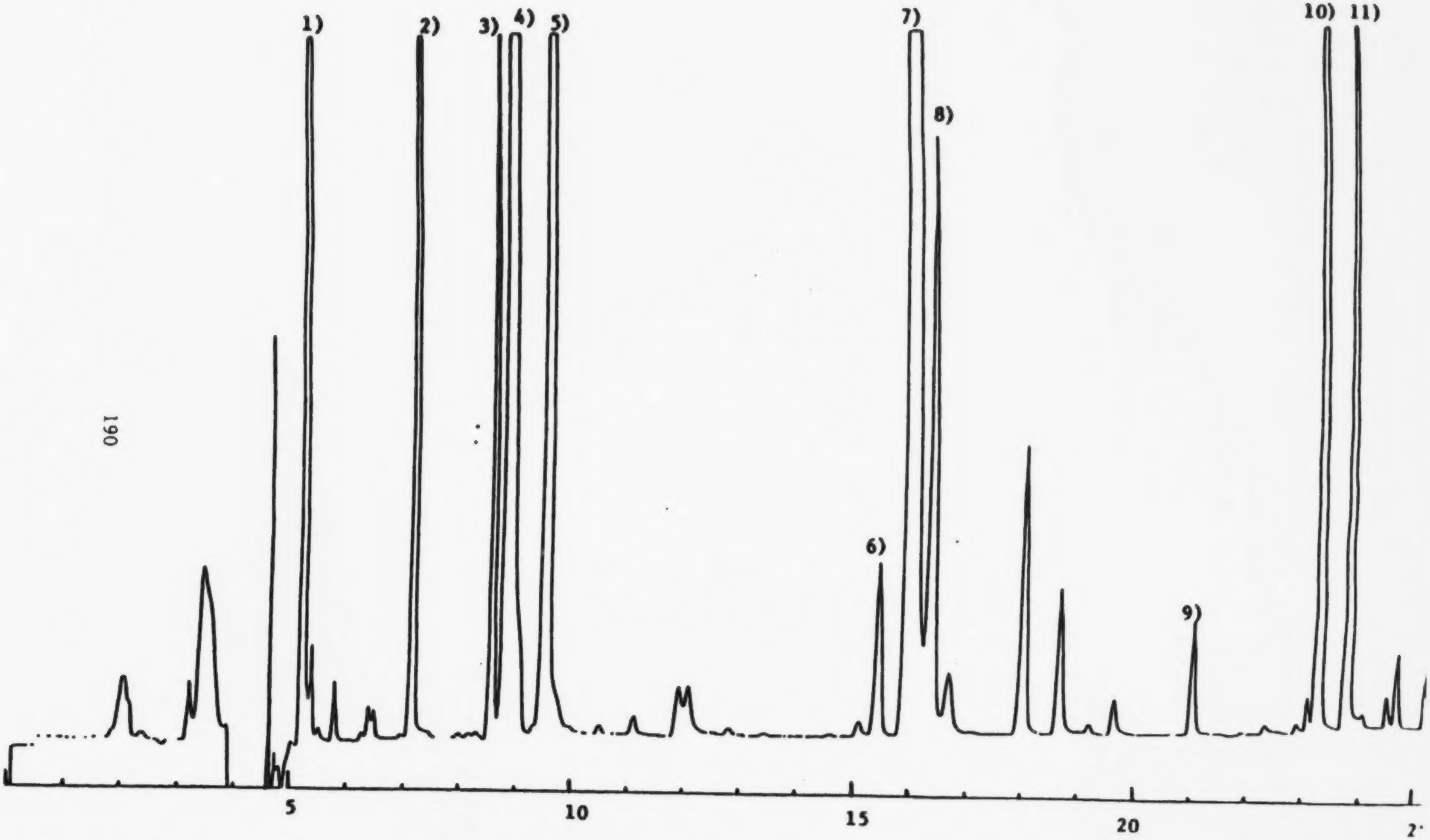


Figure 1

Reproduced from best available copy.

FIGURE 2. TAP WATER CHROMATOGRAM



1) Chloroform - 12.3 µg/L
2) MTBE Impurity - CCl_4
3) Dichloroacetonitrile - 0.5 µg/L
4) Bromodichloromethane - 30.1 µg/L
5) Chloral Hydrate - 20.0 µg/L
6) Chloropicrin - 1.2 µg/L
7) Dibromochloromethane - 30.3 µg/L
8) Bromochloroacetonitrile - 3.6 µg/L
9) 1,1,1-Trichloroacetone - 0.8 µg
10) Bromoform - 7.3 µg/L
11) Dibromoacetonitrile - 2.9 µg/L

METHOD 552. THE DETERMINATION OF HALOACETIC ACIDS IN DRINKING WATER BY LIQUID/LIQUID EXTRACTION AND GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

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METHOD 552

THE ANALYSIS OF HALOCACETIC ACIDS IN DRINKING WATER

1. SCOPE AND APPLICATION

1.1 This is a gas chromatographic (GC) method (1-4) applicable to the determination of the listed halogenated acetic acids in drinking water, ground water, raw water and any intermediate treatment stage. In addition, the chlorinated phenols listed may be analyzed by this method.

Analyte	Chemical Abstract Services Registry Number
Monochloroacetic Acid	79-11-8
Dichloroacetic Acid	79-43-6
Trichloroacetic Acid	76-03-9
Monobromoacetic Acid	79-08-3
Bromochloroacetic Acid	5589-96-3
Dibromoacetic Acid	631-64-1
2,4-Dichlorophenol	120-83-2
2,4,6-Trichlorophenol	88-06-2

1.2 This method is applicable to the determination of these analytes over the concentration ranges typically found in drinking water (1,2,4), subject to the method detection limits (MDL) listed in Table 2. The detection limits observed may vary according to the particular matrix analyzed and the specific instrumentation employed. The haloacetic acids are observed ubiquitously in chlorinated supplies at concentrations normally within the spiking level ranges in Tables 2-5.

1.3 Tribromoacetic acid has not been included because of problems with extraction and chromatography by this method. The mixed bromochloroacetic acids have recently been synthesized. The bromochloroacetic acid is present in chlorinated supplies and method validation data are provided herein. However, neat material for this compound is not readily available. The mixed trihalogenated acids may also be present. These are not included because of current problems with sample purity and the chromatography for these two compounds.

1.4 The 2-chlorophenol has not been included as a method analyte in the above list, primarily because its realistic detection limit in environmental samples is likely to be above the odor threshold. Poor precision is usually obtained for this compound at even higher levels. In addition, this analyte displays instability under the dechlorination/preservation conditions described herein. Nevertheless, some method validation data are given in Tables 2-7.

1.5 This method is designed for analysts skilled in liquid-liquid extractions, extract concentration techniques, derivatization procedures and the use of GC and interpretation of gas chromatograms.

1.6 When this method is used for the analyses of waters from unfamiliar sources, analyte identifications must be confirmed by at least one additional qualitative technique, such as GC/mass spectroscopy (MS) or by GC using dissimilar columns.

2. SUMMARY OF METHOD

2.1 A 100 mL volume of sample is adjusted to pH 11.5 and extracted with methyl-tert-butyl ether (MTBE) to remove neutral and basic organic compounds. The aqueous sample is then acidified to pH 0.5 and the acids are extracted into MTBE. After the extract is dried and concentrated, the acids are converted to their methyl esters with diazomethane (DAM). Excess DAM is removed and the methyl esters are determined by capillary GC using an electron capture detector (ECD).

3. DEFINITIONS

3.1 Internal standard -- A pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.2 Surrogate analyte -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.

3.3 Laboratory duplicates (LD1 and LD2) -- Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 Field duplicates (FD1 and FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.5 Laboratory reagent blank (LRB) -- An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 Field reagent blank (FRB) -- Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 Laboratory fortified blank (LFB) -- An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.

3.8 Laboratory fortified sample matrix (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.9 Stock standard solution -- A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.10 Primary dilution standard solution -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.11 Calibration standard (CAL) -- a solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.12 Quality control sample (QCS) -- a sample matrix containing method analytes or a solution of method analytes in a water miscible solvent which is used to fortify reagent water or environmental samples. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance with externally prepared test materials.

4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 10.2. Subtracting blank values from sample results is not permitted.

4.1.1 Glassware must be scrupulously cleaned (5). Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap water, dilute acid, and reagent water. Drain and heat in an oven or muffle furnace at 400°C for 1 hour. Do not heat volumetric ware. Thermally stable materials such as PCBs might not be eliminated by this treatment. Thorough rinsing with reagent grade acetone may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required. The extraction solvent, MTBE, may need to be redistilled.

4.2 Whereas the 2,4,6 - trichlorophenol is converted quantitatively to the corresponding anisole by the methylation procedure, (11.3), the 2,4 - dichlorophenol is only partially converted (10-20%). The 2,4 dichloroanisole partially coelutes with the 2,4,6 - trichloroanisole on the DB-1701 primary column with the chromatographic conditions employed (Table 1). The 2,4 - dichlorophenol is quantitated on the phenol peak. The extent of interference of the dichloroanisole with the 2,4,6 - trichlorophenol analysis is insignificant (0.8%) when these compounds are present at equal concentrations. For samples in which the 2,4 - dichlorophenol concentration appears significantly higher than that of the 2,4,6 - trichlorophenol, e.g. greater than a factor of 15, analyses should be performed on the DB-210 confirmation column, on which these compounds are completely resolved.

4.3 The acid forms of the analytes are strong organic acids which react readily with alkaline substances and can be lost during sample preparation. Glassware and glass wool must be acid-rinsed with (1+9) hydrochloric acid, and the sodium sulfate must be acidified (see 7.6) with sulfuric acid prior to use to avoid analyte losses due to adsorption.

4.4 Organic acids and phenols, especially chlorinated compounds, cause the most direct interference with the determination. The addition of base and subsequent extraction of the basic sample removes many neutral and basic chlorinated hydrocarbons and phthalate esters that might otherwise interfere with the electron capture analysis.

4.5 Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. Routine between-sample rinsing of the sample syringe and associated equipment with MTBE can minimize sample cross contamination. After analysis of a sample containing high concentrations of analytes, one or more injections of MTBE should be made to ensure that accurate values are obtained for the next sample.

4.6 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the water sampled. Positive identifications should be confirmed using the confirmation column specified in Table 1 or by the use of gas chromatography with mass spectrometric detection.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified (6-8) for the information of the analyst.

5.2 Diazomethane is a toxic carcinogen and can explode under certain conditions when produced in a purified or highly concentrated form. In this form, the following safety precautions must be followed.

5.2.1 Use only in a well ventilated hood -- do not breathe vapors.

5.2.2 Use a safety screen. Wear protective clothing and a shielded safety hood.

5.2.3 Use mechanical pipetting aides.

5.2.4 Do not heat above 90°C.

5.2.5 Avoid grinding surfaces, ground glass joints, sleeve bearings, glass stirrers.

5.2.6 Store away from alkali metals.

5.2.7 Solutions of diazomethane decompose rapidly in the presence of solid materials such as copper powder, calcium chloride, and boiling chips.

5.3 For the above reasons, the diazomethane generation apparatus used in the esterification procedure specified in this method (3) produces only micromolar amounts of diazomethane in very dilute solution (11.3) to minimize safety hazards. In this form, the solution is not explosive. Nevertheless, the following precautions should be followed.

5.3.1 Use only in a well ventilated hood.

5.3.2 When handling the diazomethane solution, avoid contact with skin. If contact is made, immediately wash the exposed area with warm water.

5.3.3 Collect and store the diazomethane solution produced at 0°C to minimize losses due to decomposition.

5.4 The toxicity of the extraction solvent, MTBE, has not been well defined. Susceptible individuals may experience adverse affects upon skin contact or inhalation of vapors. For such individuals a mask may be required. Protective clothing and gloves should be used and MTBE should be used only in a chemical fume hood or glove box.

6. APPARATUS AND EQUIPMENT (All specifications in Sections 6 and 7 are suggested. Catalogue numbers are provided for illustration only.)

6.1 Separatory funnels, 250 mL, with TFE fluorocarbon stopcocks, ground glass or TFE fluorocarbon stoppers.

6.2 Screw cap 40 mL vials (Pierce #13219 or equivalent). Screw caps should have TFE fluorocarbon liners.

6.3 Balance, analytical, capable of weighing to 0.0001 g.

6.4 Diazomethane generator - The generator assembly is shown in Figure 1 along with the diazomethane collection vessel.

6.5 Six or 12 position analytical concentrator. (Organomation, N-EVAP Model #111/6917 or equivalent).

6.6 Gas chromatograph - Analytical system complete with gas chromatograph equipped for electron capture detection, split/spitless capillary injection, temperature programming, differential flow control, and with all required accessories including syringes, analytical columns, gases and strip-chart recorder. A data system is recommended for measuring peak areas. An autoinjector is recommended for improved precision of analyses. The gases flowing through the election capture detector should be vented through the laboratory fume hood system.

6.7 Vials - Amber glass, 7 to 10 mL capacity with TFE-fluorocarbon lined screw cap.

6.8 Primary GC column - DB-1701 or equivalent bonded, fused silica column, 30m x 0.32mm ID, 0.25 um film thickness.

6.9 Confirmatory GC column - DB-210 or equivalent bonded, fused silica column, 30 m x 0.32 mm ID, 0.50 um film thickness.

6.10 Pasteur pipets, glass disposable, 5 3/4" length widebare diameter. (Baxter Scientific Products Giant-Pette-Pipets, Cat. No. P5240-1 or equivalent)

6.11 Volumetric ware, 5 mL.

6.12 pH Meter - Wide range with the capability of accurate pH measurements in the 0-1 and 11-12 ranges. The use of separate glass pH electrode and calomel reference electrode facilitates this measurement.

7. REAGENTS AND CONSUMABLE MATERIALS

7.1 Glass wool - Acid washed (Supelco 2-0383 or equivalent). Heat to 400°C for 1 hr.

7.2 Reagent water - Reagent water is defined as a water in which an interference is not observed at the method detection limit of each parameter of interest.

7.2.1 A Milli-pore Super-Q water system or its equivalent may be used to generate deionized reagent water. Distilled water that has been charcoal filtered may also be suitable.

7.2.2 Test reagent water each day it is used by analyzing according to Sect. 11.

7.3 Methanol - Pesticide quality or equivalent.

7.4 Ethyl ether - (Burdick and Jackson Product 107 or equivalent). Nanograde, redistilled in glass if necessary. Ethers must be free of peroxides as indicated by EM Quant test strips, available from EM Science, Gibbstown, NJ. Procedures recommended for removal of peroxides are provided with the test strips. Ethers must be periodically tested (monthly) for peroxide formation during use.

7.5 Methyl-tert-butyl ether - (EM Sciences Omnisolve High Purity Grade Solvent or equivalent). Nanograde, redistilled in glass if necessary. The same peroxide precautions as in 7.4 apply to this ether.

7.6 Sodium sulfate - (ACS) granular, acidified, anhydrous. Heat in a shallow tray at 400°C for a minimum of 4 hr to remove phthalates and other interfering organic substances. Alternatively, extract with methylene chloride in a Soxhlet apparatus for 48 hr. Acidify by slurrying 100 g sodium sulfate with just enough ethyl ether to cover the solid. Add 0.1 mL concentrated sulfuric acid and mix thoroughly. Remove the ether under vacuum or allow to evaporate in a loosely covered beaker in a hood. Mix 1 g of the resulting solid with 5 mL of reagent water and measure the pH of the mixture. It must be below pH 4. Store at 130°C.

7.7 Sulfuric acid solution (1+1) - Slowly add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

7.8 Sodium hydroxide (NaOH), 1N - Dissolve 4 g ACS grade in reagent water and dilute up to 100 mL in a 100 mL volumetric flask.

7.9 Potassium Hydroxide (KOH), 37% - Dissolve 37 g of ACS grade in reagent water and dilute up to 100 mL in a 100 mL volumetric flask.

7.10 Carbitol - (Diethylene glycol monoethyl ether), ACS. Available from Aldrich Chemical Co.

7.11 Diazald - (N-methyl-N-nitroso-p-toluenesulfonamide), ACS. Available from Aldrich Chemical Co.

7.12 Diazald Solution - Prepare a solution containing 10 g Diazald in 100 mL of a 50:50 by volume mixture of ethyl ether and carbitol. This solution is stable for one month or longer when stored at 4°C in an amber colored bottle with a teflon-lined screw cap.

7.13 Silica Gel - Chromatographic grade, nominal 100 mesh. Heat to 400°C for 4 hr. Store at 130°C.

7.14 Acetone - ACS reagent grade or equivalent.

7.15 Ammonium Chloride - ACS reagent grade or equivalent.

7.16 Sodium Sulfite - ACS reagent grade or equivalent.

7.17 1,2,3-Trichloropropane, Aldrich Chemical, 99+%.

7.18 3,5-Dichlorobenzoic Acid, Aldrich Chemical, 99%.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Grab samples must be collected in accordance with conventional sampling practices (9) using glass containers with TFE-lined screw-caps and capacities in excess of 100 mL.

8.1.1 To combine residual chlorine, add ammonium chloride (NH_4Cl) to the sample container in an amount to produce a concentration of 100 mg/L in the sample. Add 0.1 mL of a 10 mg/mL aqueous solution of NH_4Cl to the sample bottle for each 100 mL of sample bottle capacity at the sample site or in the laboratory prior to shipment to the sampling site.

8.1.2 After collecting the sample in the bottle containing the dechlorination reagent, seal the bottle and agitate for 1 minute.

8.1.3 Samples must be iced or refrigerated at 4°C and maintained at these conditions away from light until extraction. Holding studies performed to date have suggested that, in samples dechlorinated with NH_4Cl, the analytes are stable for up to 28 days. Since stability may be matrix dependent, the analyst should verify that the prescribed preservation technique is suitable for the samples under study.

8.1.4 Alternatively, the samples may be dechlorinated with 100 mg/L of sodium sulfite (Na_2SO_3). Limited holding studies performed to date have suggested that 2,4-dichlorophenol and monobromoacetic acid may show evidence of decomposition beyond seven days in the presence of Na_2SO_3.

8.1.5 Dried extract concentrates (11.3.7) should be stored at 4°C away from light in glass vials with TFE-faced caps. Extracts should be analyzed within 24 hours following preparation.

9. CALIBRATION

9.1 Establish GC operating parameters equivalent to specifications in Table 1. The GC system must be calibrated using the internal standard (IS) technique.

9.2 Internal Standard Calibration Procedure - This approach requires the analyst to select one or more internal standards which are compatible in analytical behavior with the method analytes. For the single laboratory precision and accuracy data reported in Tables 2-7, one internal standard (1,2,3-trichloropropane) was employed. The concentration of the internal standard used in obtaining these data was 0.4 µg/mL in the final 5.0 mL concentrate (11.3.4).

9.2.1 Prepare separate stock calibration standards for each compound of interest at a concentration of 1-5 mg/mL in MTBE solvent. Method analytes may be obtained as neat materials (>99% purity) from a number of commercial suppliers or as ampulized solutions from the EPA Repository for Hazardous Materials.

9.2.2 To prepare working standards, combine and dilute stock calibration standards with MTBE. The working standards must comprise at least three concentration levels (optimally five) of each analyte with the lowest standard being at or near the method detection limit of each analyte. The concentrations of the other standards should define a range containing the expected sample concentrations or the working range of the detector.

9.2.3 Esterify 4 mL aliquots of each working calibration standard with diazomethane as described in Sections 11.3.1 - 11.3.7. NOTE that the concentration of the working standard must be corrected for the dilution from 4 mL to 5 mL required in 11.3.3.

9.2.4 Inject 2 uL of each standard and calculate the relative response for each analyte (RR_a) using the equation:

$$RR_a = A_a/A_{is}$$

Where A_a is the peak area of the analyte and A_{is} the peak area of the internal standard.

9.2.5 Generate a calibration curve of RR_a versus analyte concentration of the standards expressed in equivalent µg/L in the original (100 mL) aqueous sample. The working calibration curve must be verified daily by measurement of one or more calibration standards. If the response for any analyte falls outside the predicted response by more than 15%, the calibration check must be repeated using a freshly prepared calibration standard. Should the retest fail, a new calibration curve must be generated.

10. QUALITY CONTROL

10.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, determination of surrogate compound recoveries in each sample and blank, monitoring internal standard peak area or height in each sample and blank, analysis of laboratory reagent blanks, laboratory fortified blanks, laboratory fortified sample matrices, and QC samples. Additional quality control practices are recommended.

10.2 LABORATORY REAGENT BLANKS (LRB). Before processing any samples, the analyst must analyze at least one LRB to demonstrate that all glassware and reagent interferences are under control. In addition, each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window (11.4.4) of any analyte, the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

10.3 INITIAL DEMONSTRATION OF CAPABILITY

10.3.1 Select a representative fortified concentration for each of the target analytes. Concentrations near level 3 (Table 4) are recommended. Prepare a laboratory control (LC) sample concentrate in methanol 1000 times more concentrated than the selected concentration. With a syringe, add 100 uL of the LC sample concentrate to each of four to seven 100 mL aliquots of reagent water. Analyze the aliquots according to the method beginning in Section 11 and calculate mean recoveries and standard deviation for each analyte.

10.3.2 Calculate the mean percent recovery (R) and the standard deviation of the recovery (S_R). For each analyte, the mean recovery values for all must fall in the range of R ± 30% (or within R ± $3S_R$ if broader) using the values for R and S_R for reagent water in Table 4. The standard deviation should be less than ± 30% or $3S_R$, whichever is larger. For those compounds that meet these criteria, performance is considered acceptable and sample analysis may begin. For those compounds that fail these criteria, this procedure must be repeated using a minimum of five fresh samples until satisfactory performance has been demonstrated.

10.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. It is expected that as laboratory personnel gain experience with this method, the quality of data will improve beyond those required here.

10.3.4 The analyst is permitted to modify GC columns, GC conditions, detectors, extraction techniques, concentration techniques (i.e., evaporation techniques), internal standard or surrogate compounds. Each time such method modifications are made, the analyst must repeat the procedures in Sect. 10.3.1.

10.4 ASSESSING SURROGATE RECOVERY

10.4.1 When surrogate recovery from a sample or method blank is <70% or >130%, check (1) calculations to locate possible errors, (2) standard solutions for degradation, (3) contamination, and (4) instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract.

10.4.2 If the extract reanalysis fails the 70-130% recovery criterion, the problem must be identified and corrected before continuing.

10.4.3 If the extract reanalysis meets the surrogate recovery criterion, report only data for the analyzed extract. If sample extract continues to fail the recovery criterion, report all data for that sample as suspect.

10.4.4 Develop and maintain control charts on surrogate recovery as described in 10.6.2. Charting of surrogate recoveries is an especially valuable activity, since these are present in every sample and the analytical results will form a significant record of data quality.

10.5 ASSESSING THE INTERNAL STANDARD

10.5.1 When using the internal standard calibration procedure, the analyst is expected to monitor the IS response (peak area or peak height) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from daily calibration standard's IS response by more than 30%.

10.5.2 If >30% deviation occurs with an individual extract, optimize instrument performance and inject a second aliquot of that extract.

10.5.2.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.

10.5.2.2 If a deviation of greater than 30% is obtained for the reinjected extract, analysis of the samples should be repeated beginning with Sect. 11, provided the sample is still available. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

10.5.3 If consecutive samples fail the IS response acceptance criterion, immediately analyze a calibration check standard.

10.5.3.1 If the check standard provides a response factor (RF) within 20% of the predicted value, then follow procedures itemized in Sect. 10.5.2 for each sample failing the IS response criterion.

10.5.3.2 If the check standard provides a response factor which deviates more than 20% of the predicted value, then the analyst must recalibrate, as specified in Sect. 9.

10.6 LABORATORY FORTIFIED BLANK

10.6.1 The laboratory must analyze at least one laboratory fortified blank (LFB) sample with every 20 samples or one per sample set (all samples extracted within a 24-hr period), whichever is greater. Fortified concentrations near level 3 (Table 4) are recommended. Calculate accuracy as percent recovery (R). If the recovery of any analyte falls outside the control limits (see Sect. 10.6.2), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

10.6.2 Prepare control charts based on mean upper and lower control limits, $R \pm 3 S_R$. The initial demonstration of capability (10.3) establishes the initial limits. After each four to six new recovery measurements, recalculate R and S_R using all the data, and construct new control limits. When the total number of data points reach 20, update the control limits by calculating R and S_R using only the most recent 20 data points. At least quarterly, replicates of LFBs should be analyzed to determine the precision of the laboratory measurements. Add these results to the ongoing control charts to document data quality.

10.7 LABORATORY FORTIFIED SAMPLE MATRIX

10.7.1 The laboratory must add known concentrations of analytes to a minimum of 10% of the routine samples or one concentration per sample set, whichever is greater. The concentrations should be equal to or greater than the background concentrations in the sample selected for fortification. Ideally, the concentration should be the same as that used for the laboratory fortified blank (Sect. 10.6). Over time, samples from all routine sample sources should be fortified.

10.7.2 Calculate the mean percent recovery, R, of the concentration for each analyte, after correcting the total mean measured concentration, A, from the fortified sample for the back-ground concentration, B, measured in the unfortified sample, i.e.:

$$R = 100 (A - B) / C,$$

where C is the fortifying concentration. Compare these values to control limits appropriate for reagent water data collected in the same fashion (10.6).

10.7.3 If the analysis of the unfortified sample reveals the absence of measurable background concentrations, and the added concentrations are those specified in Sect. 10.6, then the appropriate control limits would be the acceptance limits in Sect. 10.6.

10.7.4 If the sample contains measurable background concentrations of analytes, calculate mean recovery of the fortified concentration, R, for each such analyte after correcting for the background concentration.

$$R = 100\ (A - B)/C$$

Compare these values to reagent water recovery data, R*, at comparable fortified concentrations from Tables 3-5. Results are considered comparable if the measured recoveries fall within the range,

$$R^* \pm 3S_c,$$

where S_c is the estimated percent relative standard deviation in the measurement of the fortified concentration. By contrast to the measurement of recoveries in reagent water (10.6.2) or matrix samples without background (10.7.3), the relative standard deviation, S_c, must be expressed as the statistical sum of variation from two sources, the measurement of the total concentration as well as the measurement of background concentration. In this case, variances, defined as S^2, are additive and Sc can be expressed as

$$S_c^2 = S_a^2 + S_b^2$$

or $$S_c = (S_a^2 + S_b^2)^{1/2},$$

where Sa and Sb are the percent relative standard deviations of the total measured concentration and the background concentration respectively. The value of Sa may be estimated from the mean measurement of A above or from data at comparable concentrations from Tables 3-5. Likewise, S_b so can be measured from repetitive measurements of the background concentration or estimated from comparable concentration data from Tables 2-5.

10.7.5 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Sect. 10.6), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

10.8 QUALITY CONTROL SAMPLE (QCS) - At least quarterly, analyze a QCS from an external source. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem source.

10.9 The laboratory may adapt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

11. PROCEDURE

11.1 SAMPLE CLEANUP AND EXTRACTION

11.1.1 Remove the samples from storage (Sect. 8.1.3) and allow to equilibrate to room temperature.

11.1.2 Transfer 100 mL of sample with a pipet to a 250 mL separatory funnel. Add 1 mL of 1.0 N NaOH solution. Remove an aliquot and measure pH, which should be approximately 11.5.

11.1.3 Return aliquot to separatory funnel. (NOTE: If sufficient sample is available, use a separate 100 mL sample to measure the basic pH and to determine the amount of H_2SO_4 required in 11.1.4.) The measurement of pH here and in 11.1.4 should be done with the wide range pH meter described in 6.12. Add 30 mL MTBE. Extract the sample one time by vigorously shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Discard the organic phase and return the aqueous phase to the 250 mL separatory funnel.

11.1.4 Add sufficient 1:1 H_2SO_4 in reagent water (ca. 15-20 mL) to adjust the pH to pH $\leq$ 0.5. Add 15 mL of MTBE and extract for 2 minutes as in 11.1.3. Allow the phases to separate for 10 min. If an emulsion persists employ the same procedures for separation as in 11.1.3. Separate the phases and collect the MTBE phase in a 40 mL screw cap vial (6.2). Add 15 mL of MTBE to the sample and repeat the extraction a second time. Combine the extracts in the 40 mL vial.

11.2 EXTRACT CONCENTRATION AND DRYING TECHNIQUES

11.2.1 Extract Concentration - Evaporate the solvent at room temperature to a volume of 1-2 mL under a gentle stream of dry nitrogen. Under these conditions the sample cools during evaporation and some condensation will be observed on the outside of the vial. Alternately place the vials in a water bath maintained at 35°C and concentrate to a minimum volume of 2 mL. The method validation data in Tables 2-7 were obtained with the former technique. These gentle concentration conditions are necessary because of the volatility of the monohaloacetic acids.

11.2.2 Extract Drying Technique

11.2.2.1 Prepare sodium sulfate drying tubes by inserting a small piece of acid washed glass wool into the bottom, restricted, end of a wide bore, Pasteur pipet (6.10).

11.2.2.2 Add a column of approximately 5 cm acidified sodium sulfate. Tap pipet gently to pack sodium sulfate.

11.2.2.3 Immediately, using another Pasteur pipet, transfer the 1-2 mL MTBE extract from the 40 mL vial into the top of the drying tube. A small amount of separated water phase will likely be present in the bottom of the 40 mL vial. Avoid transferring any of the water phase. Examine the lower portion of the Pasteur pipet to see whether a separate water phase is present. Collect dried extract in a 5.0 mL volumetric flask.

11.2.2.4 Rinse sides of 40 mL sample tube with approximately 0.7 mL of clean MTBE. Transfer this MTBE into the drying tube using the same pipet as in Step 3.

11.2.2.5 Repeat Step 4 until the volumetric flask contains 3.8 to 4.0 mL.

11.3 ESTERIFICATION OF ACIDS

11.3.1 Assemble the diazomethane generator shown in Figure 1 in a hood. The collection vessel is a 10-15 mL vial, equipped with a teflon-lined screw cap and maintained at 0-5°C.

11.3.2 Add a sufficient amount of ethyl ether to tube 1 to cover the first impinger. Add 5 mL of MTBE to the collection vial. Set the nitrogen flow at 5-10 cm^3/min. Add 2 mL Diazald solution and 1.5 mL of 37% KOH solution to the second impinger. Connect the tubing as shown and allow the N_2 flow to purge the diazomethane from the reaction vessel into the collection vial for 30 min. Cap the vial when collection is complete and maintain at 0-5°C. When stored at 0-5°C this diazomethane solution may be used over a period of 48 hours.

11.3.3 To each flask containing sample or working standard (9.2.3), add 100 ul methanol and 500 ul of diazomethane solution. A persistent yellow color after the addition of diazomethane indicates that an excess of diazomethane was available for esterification of the analytes. If this is not obtained, then continue adding successive 50 ul aliquots of diazomethane solution until the persistent yellow color is achieved. Dilute to a final volume of 5.0 mL with MTBE.

11.3.4 Spike the samples and working standards with identical volumes of the internal standard(s). The appropriate amount of internal standard is dependent on the calibration range. As a general rule, the internal standard response should be approximately equal to the response produced by the middle trichloroacetic acid calibration standard. For the validation data in Tables 3-7, 20 µl of a 100 µg/mL internal standard solution in MTBE were added to the 5.0 mL concentrate to yield a concentration of 0.4 µg/mL.

11.3.5 Allow the sample to remain in contact with diazoamethane for 30 minutes. Remove any unreacted diazomethane by addition of 0.2 g silica gel. Effervescence due to nitrogen evolution is a further indication that excess diazomethane is present.

11.3.6 Seal the flasks with teflon or glass stoppers and store in the hood at room temperature for 15 min.

11.3.7 The samples are now ready for analysis. Analyze as soon as possible. The samples may be stored in the stoppered flasks at 0-4°C for 24 hours if the analysis cannot be performed immediately.

11.4 GAS CHROMATOGRAPHY

11.4.1 Table 1 summarizes the recommended operating conditions for the GC. Included in Table 1 are the retention times observed using this method. An example of the separation achieved using these conditions is shown in Figure 2. Other GC columns, chromatographic conditions, or detectors may be used if the requirements of Section 10.3 are met.

11.4.2 Calibrate the system daily as described in Section 9. The standards and extracts must be in MTBE.

11.4.3 Inject 2 ul of the sample extract. Record the resulting peak size in area units.

11.4.4 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.4.5 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12. CALCULATIONS

12.1 Calculate analyte concentrations in the sample from the response for the analyte relative to the internal standard (RR_a) using the equation in Section 9.2.4.

12.2 For samples processed as part of a set where recoveries falls outside of the control limits established in Section 10, results for the affected analytes must be labeled as suspect.

13. PRECISION AND ACCURACY

13.1 In a single laboratory (EMSL-Cincinnati), recovery and precision data were obtained at four concentrations in reagent water (Tables 2-5). Tables 6 and 7 give representative recovery and precision data for fortified tap water, which had been chlorinated. The Method Detection Limit (MDL) (10) data are given in Table 2, and Tables 3-5 illustrate instrument range. In the calculation of MDL's, the mean observed concentrations were not corrected for recovery.

14. REFERENCES

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TABLE 1. RETENTION DATA AND CHROMATOGRAPHIC CONDITIONS

Analyte	Retention Time, min. Column A	 Column B
Monochloroacetic Acid	5.77	10.97
Monobromoacetic Acid	8.70	13.03
Dichloroacetic Acid	9.40	12.72
Trichloroacetic Acid	12.20	14.37
1,2,3-Trichloropropane a)	13.28	13.87
Bromochloroacetic Acid	13.52	15.11
Dibromoacetic Acid	16.00	16.83
2-Chlorophenol	16.65	18.32
2,4-Dichlorophenol	20.70	19.27
2,4,6-Trichlorophenol	21.94	22.08
3,5-Dichlorobenzoic Acid b)	23.06	23.95

Column A: DB-1701, 30 m x 0.32 mm i.d., 0.25 µm film thickness, Injector Temp. = 200°C, Detector Temp. = 290°C, Linear Helium Flow = 27 cm/sec, Splitless injection with 30 s delay

Program: Hold at 50°C for 10 min, to 210°C at 10°C/min. and hold 10 min.

Column B: DB-210, 30 m x 0.32 mm i.d., 0.50 µm film thickness, Injector Temp. = 200°C, Detector Temp. = 290°C, Linear Helium Flow = 25 cm/sec, splitless injection with 30 s delay.

Program: Hold at 50°C for 10 min., to 210°< at 10°C/min and hold 10 min.

(a) Internal Standard

(b) Surrogate Acid

TABLE 2. ANALYTE RECOVERY AND PRECISION DATA AND METHOD DETECTION LIMITS[a]

LEVEL 1 IN REAGENT WATER

Analyte	Fortified Conc. µg/L	Mean Meas. Conc. µg/L	Std. Dev. µg/L	Rel. Std. Dev.,%	Mean Recovery %	Method Detection Limit µg/L
Monochloroacetic Acid	0.050	0.037	0.014	38	74	0.052
Monobromoacetic Acid	0.050	0.029	0.002	7	58	0.0074
Dichloroacetic Acid	0.050	0.042	0.004	10	84	0.015
Trichloroacetic Acid	0.050	0.039	0.023	59	78	0.085
Bromomonochloroacetic acid	0.100	0.150	0.063	41	150	0.14
Dibromoacetic Acid	0.050	0.029	0.004	14	58	0.015
2-Chlorophenol	0.200	0.123	0.038	31	61	0.14
2,4-Dichlorophenol	0.250	0.147	0.085	58	59	0.32
2,4,6-Trichlorophenol	0.050	0.033	0.006	18	66	0.022

[a] Produced by analysis of seven aliquots of fortified reagent water (Reference 10).

TABLE 3. ANALYTE RECOVERY AND PRECISION DATA[a]

LEVEL 2 IN REAGENT WATER

Analyte	Fortified Conc. µg/L	Mean Meas. Conc. µg/L	Std. Dev. µg/L	Rel. Std. Dev., %	Mean Recovery %
Monochloroacetic Acid	1.0	0.81	0.065	8	81
Monobromoacetic Acid	1.0	0.61	0.046	8	61
Dichloroacetic Acid	2.5	2.53	0.15	6	101
Trichloroacetic Acid	0.50	0.30	0.032	11	60
Bromochloroacetic Acid	0.50	0.51	0.041	8	103
Dibromoacetic Acid	1.25	0.81	0.033	4	65
2-Chlorophenol	2.50	1.79	0.62	35	72
2,4-Dichlorophenol	1.00	0.74	0.072	10	74
2,4,6-Trichlorophenol	0.50	0.43	0.032	7	86

[a] Produced by the analysis of seven aliquots of fortified reagent water.

TABLE 4. ANALYTE RECOVERY AND PRECISION DATA[a]

LEVEL 3 IN REAGENT WATER

Analyte	Fortified Conc. µg/L	Mean Meas. Conc. µg/L	Std. Dev. µg/L	Rel. Std. Dev., %	Mean Recovery %
Monochloroacetic Acid	5.0	3.47	0.25	7	69
Monobromoacetic Acid	5.0	2.85	0.13	5	57
Dichloroacetic Acid	12.50	11.84	0.25	2	95
Trichloroacetic Acid	2.50	2.18	0.083	4	87
Bromochloroacetic Acid	1.00	0.90	0.059	7	90
Dibromoacetic Acid	2.50	1.84	0.11	6	74
2-Chlorophenol	6.25	5.66	0.34	6	91
2,4-Dichlorophenol	5.00	5.12	0.47	9	102
2,4,6-Trichlorophenol	2.50	2.47	0.054	2	99

[a] Produced by the analysis of seven aliquots of fortified reagent water.

TABLE 5. ANALYTE RECOVERY AND PRECISION DATA[a]

LEVEL 4 IN REAGENT WATER

Analyte	Fortified Conc. µg/L	Mean Meas. Conc. µg/L	Std. Dev. µg/L	Rel. Std. Dev., %	Mean Recovery %
Monochloroacetic Acid	10.0	7.08	0.16	2.3	71
Monobromoacetic Acid	10.0	7.62	0.18	2.4	76
Dichloroacetic Acid	25.0	24.1	0.41	1.7	96
Trichloroacetic Acid	5.00	5.70	0.11	1.9	114
Bromochloroacetic Acid	5.00	4.66	0.22	4.7	93
Dibromoacetic Acid	5.00	5.35	0.096	1.8	107
2-Chlorophenol	12.50	12.7	0.66	5.2	102
2,4-Dichlorophenol	10.00	11.0	0.57	5.2	110
2,4,6-Trichlorophenol	5.00	5.18	0.072	1.4	104

[a] Produced by the analysis of seven aliquots of fortified reagent water.

TABLE 6. ANALYTE RECOVERY AND PRECISION DATA[a]

LEVEL 1 IN TAP WATER

Analyte	Back-ground Conc., µg/L	Fortified Conc. µg/L	Mean[b] Meas. Conc. µg/L	Std. Dev. µg/L	Rel. Std. Dev. %	Mean Recovery %
Monochloroacetic Acid	1.83	3.60	2.23	0.19	8	62
Monobromacetic Acid	0.32	1.20	1.36	0.11	8	113
Dichloroacetic Acid	32.3	36.0	26.0	2.4	9	72
Trichloroacetic Acid	5.4	10.0	10.7	0.83	8	107
Dibromoacetic Acid	10.6	15.0	19.2	1.4	7	128
2-Chlorophenol	11.5	45.0	41.6	8.5	20	92
2,4-Dichlorophenol	0	10.0	12.0	1.2	10	120
2,4,6-Trichlorophenol	0	2.00	28.8	0.23	8	144

[a] Produced by the analysis of seven aliquots of fortified tap water.

[b] Background level subtracted.

TABLE 7. ANALYTE RECOVERY AND PRECISION DATA[a]

LEVEL 2 IN TAP WATER

Analyte	Background Conc. µg/L	Fortified Conc. µg/L	Mean[b] Meas. Conc. µg/L	Std. Dev. µg/L	Rel. Std. Dev. %	Mean Recovery %
Monochloroacetic Acid	1.44	10.0	6.45	0.26	40	64
Monobromacetic Acid	0.27	4.00	3.85	0.20	5	96
Dichloroacetic Acid	27.9	72.0	61.0	2.9	5	85
Trichloroacetic Acid	49.2	20.0	20.7	1.0	5	104
Dibromoacetic Acid	11.0	30.0	34.1	0.89	3	114
2-Chlorophenol	11.0	60.0	69.8	6.0	9	116
2,4-Dichlorophenol	0	30.0	26.9	1.5	55	90
2,4,6-Trichlorophenol	0	10.0	10.7	0.27	2	107

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[a] Produced by the analysis of seven aliquots of fortified tap water.

[b] Background level subtracted.

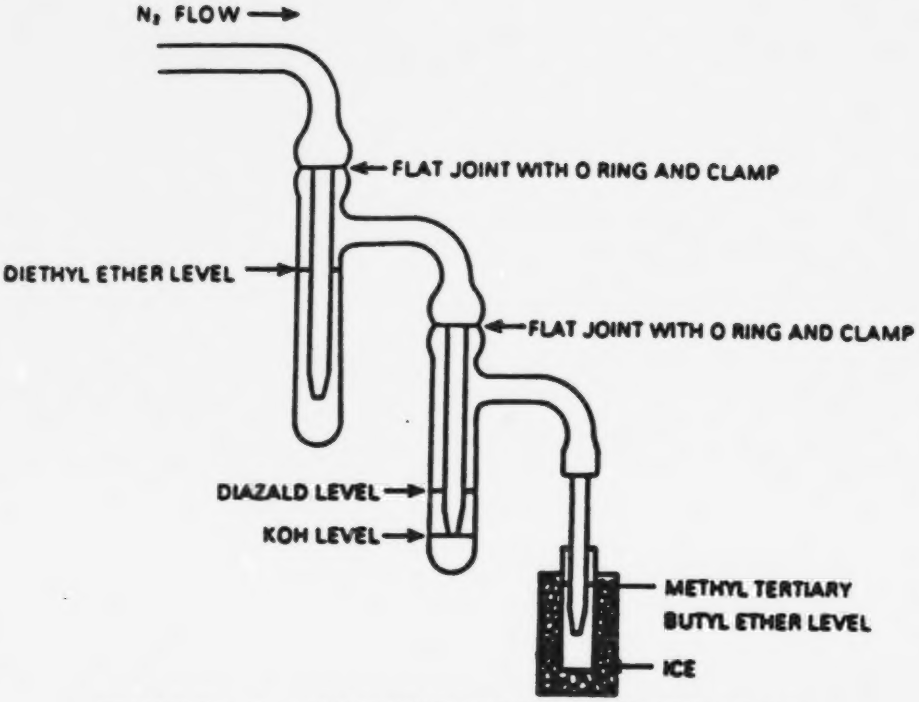
N₂ FLOW
FLAT JOINT WITH O RING AND CLAMP
DIETHYL ETHER LEVEL
FLAT JOINT WITH O RING AND CLAMP
DIAZALD LEVEL
KOH LEVEL
METHYL TERTIARY BUTYL ETHER LEVEL
ICE

FIGURE 1. DIAZOMETHANE GENERATOR

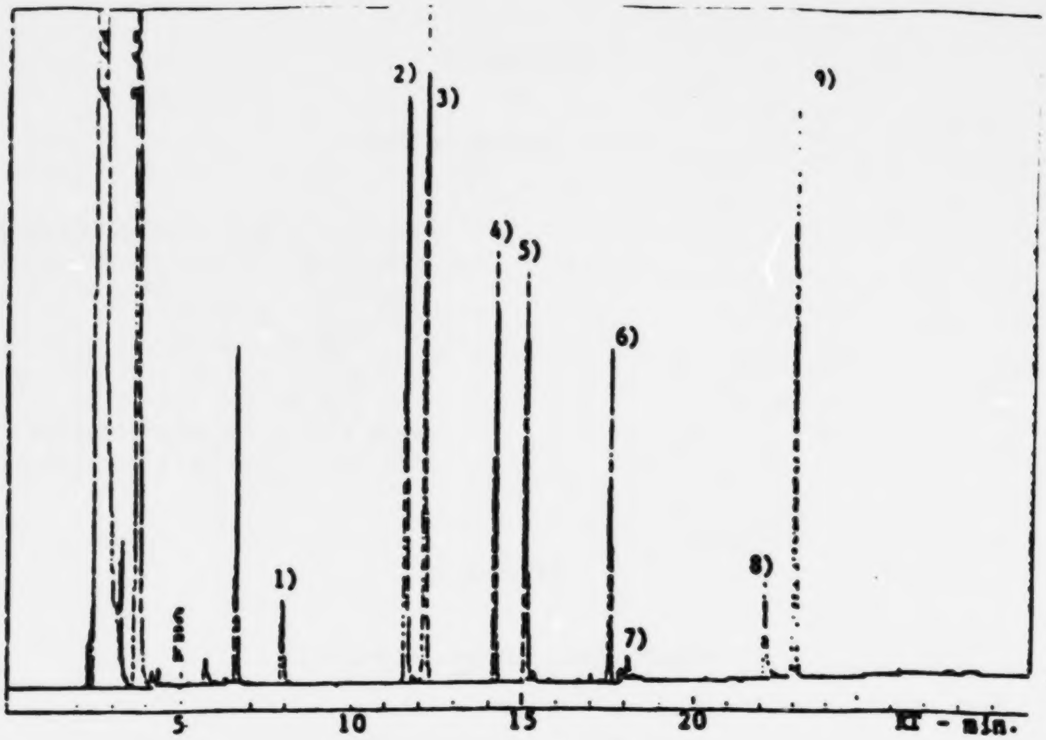

FIGURE 2A

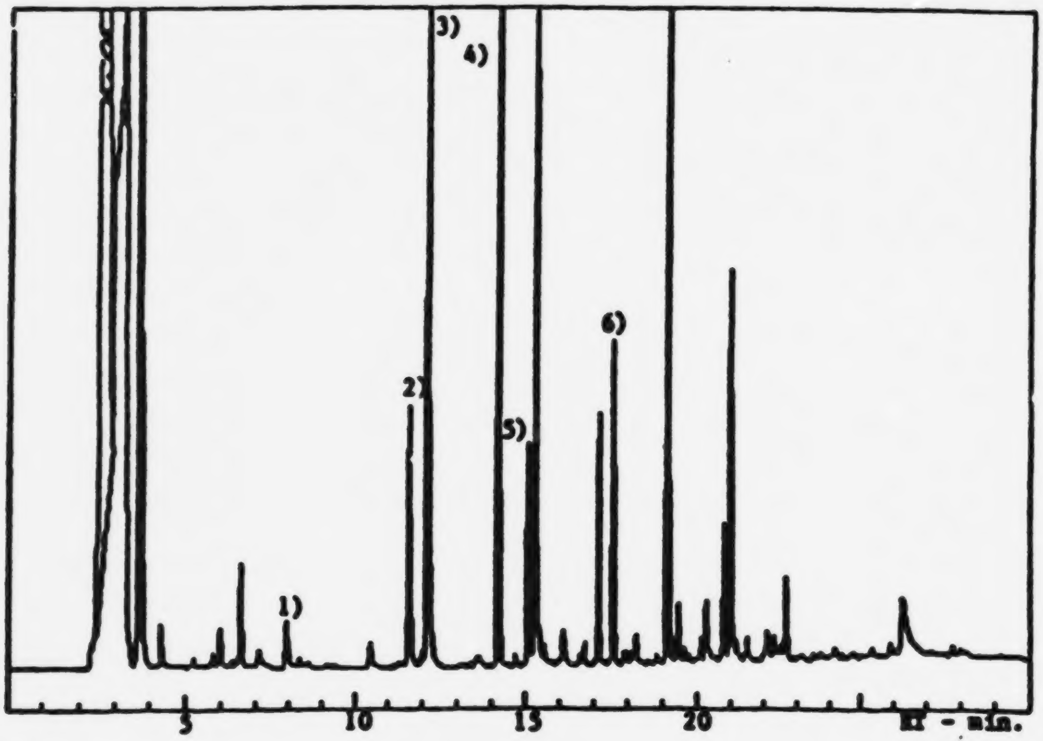

FIGURE 2B

FIGURE 2A

Spiked Reagent Water

1) Monochloracetic Acid - 6.25 µg/L
2) Monobromacetic Acid - 6.25 µg/L
3) Dichloroacetic Acid - 6.25 µg/L
4) Trichloroacetic Acid - 1.6 µg/L
5) Internal Standard - 20 µg/L
6) Dibromoacetic Acid - 1.0 µg/L
7) 2-Chloro-Phenol - 6.25 µg/L
8) 2, 4-Dichlorophenol - 6.25 µg/L
9) 2,4,6-Trichlorophenol - 1.6 µg/L

FIGURE 2B

Representative Tap Water Sample

1) Monochloroacetic Acid - Background + 5 µg/L Spike
2) Monobromo-acetic Acid - Background + 5 µg/L Spike
3) Dichloroacetic Acid - 13.4 µg/L
4) Trichloroacetic Acid - 3.7 µg/L
5) Internal Standard - 20 µg/L
6) Dibromoacetic Acid - 2.0 µg/L